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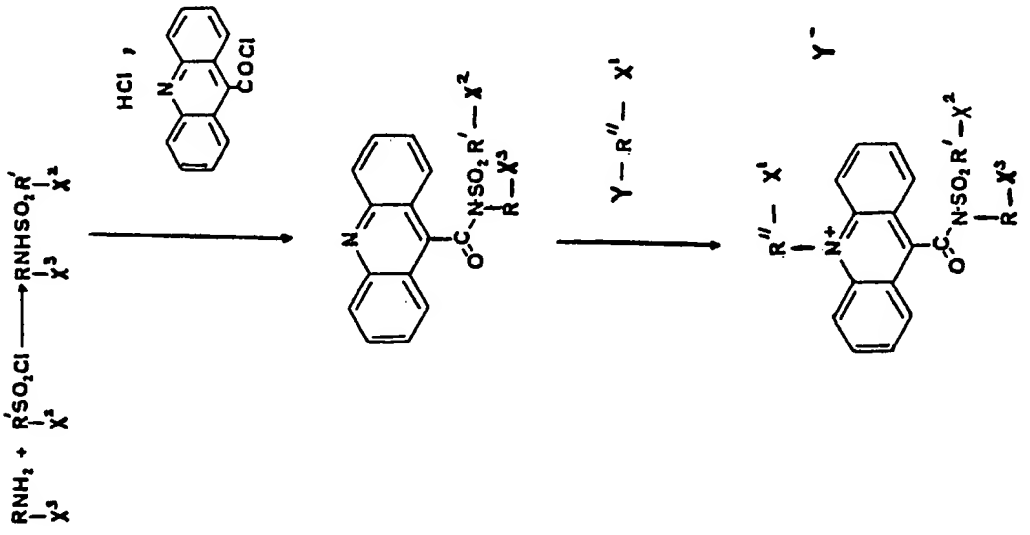
(54) Chemiluminescent acridinium and phenantridinium salts.

(57) Acridinium sulfonylamides and isomers, such as phenanthridinium sulfonylamides, may be employed in applications including chemiluminmescent immunoassays. Methods for synthesis of these compounds include contacting an amine with a sulfonylhalide to form a sulfonamide and acylating with an activated carboxylic acid of an acridine or isomer thereof. The N-sulfonyl-9-acridinium carboxamide and isomer may be conjugated to antigens, haptens, antibodies, and nucleic acids for use in chemiluminescent assays.



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CHEMILUMINESCENT ACRIDINIUM SALTS

Background

The present invention relates in general to chemiluminescent methods and materials and in particular to methods and materials involving chemiluminescent acridinium and phenanthridinium salts.

Chemiluminescence may be defined as the generation of light from a chemical reaction. The mechanism of most chemiluminescent reactions is not known in detail, but a generalized mechanism [Schuster et al., Advances in Physical Organic Chemistry, 187-238 (1984)] may be outlined:

$A \rightarrow B^* \rightarrow B + h\nu$

Compound A undergoes a chemical reaction (usually oxidation) to yield a product in an electronically excited State ("B*"). As it returns to the ground state ("B"), this product gives up energy in the form of light ("hν").

Although competing dark reactions may decrease the efficiency of the overall reaction to less than 1%, some bioluminescent systems may achieve 60-70% efficiency, and, in many cases, limits of detection in the femtomole (10^{-15} mole) to attomole (10^{-18} mole) range have been recorded.

Chemiluminescence has been used for a variety of purposes in analytical chemistry where other methods fail to have adequate sensitivity. In immunodiagnostics, chemiluminescent immunoassays ("CLIA") may thus match or exceed the sensitivity of radioimmunoassays ("RIA") or enzyme immunoassays ("EIA") [Kircka et al., Diagnostic Medicine , 1, 45-52 (1984)].

Luminol and isoluminol derivatives are the most widely used chemiluminescent reagents for immunoassays. The light-yielding reaction is initiated by oxidation with alkaline hydrogen peroxide in the presence of catalysts such as microperoxidase or transition metal ions. Light emission occurs at about 465 nm, which corresponds to the fluorescence emission of the product, aminophthalic acid. Aminobutylethyl isoluminol ("ABEI") may be used as a label in immunoassays and is commercially available.

A second group of chemiluminescent reagents, aryl oxalates [Gill, Aldrichimica Acta, 16 , 59-61 (1983) and Catherall et al., J. Chem. Soc. Faraday Trans. 2, 80, 823-834 (1984)], have been used as commerical cold light sources [see e.g., Tseng et al., U.S. Patent No. 4,338,213] and in high performance liquid chromatography ("HPLC") detectors [Kobayashi et al., Anal. Chem., 52, 424-427 (1980) and Miyaguchi et al., J. Chromatogr., 303, 173-176 (1984)]. It is thought that these derivatives react with hydrogen peroxide in buffered or unbuffered solvents to give a dioxetan-dione which decomposes quickly to give CO_2 in an excited state. Energy is then transferred by electron transfer to a fluorescer molecule which emits light.

A third group of reagents, 10-methyl-acridinium-9-carboxylic acid aryl esters, are chemiluminescent in the presence of alkaline hydrogen peroxide and in the absence of a catalyst. The mechanism is thought to involve initial attack by a hydroperoxide anion, followed by intramolecular displacement of the phenolate (the "leaving group") to give a strained dioxetan-one. The strained dioxetan-one decomposes to CO_2 and excited N-methyl-acridone, which emits light at 430 nm. Carboxy-substituted acridinium salts have been used as labels in immunoassays [Weeks et al., Clin. Chem., 29, 1474-79 (1983); Campell et al., European Patent Application No. 82,636; and McCapra et al., UK Patent No. GB 1,461,877]. Also, 5-methyl-phenanthridinium-6-carboxylic acid aryl esters, which are isomeric with the acridinium aryl esters, have been used as labels in immunoassays [Lin et al, European Patent Application No. 170,415].

Despite their usefulness in immunoassays, antibody-conjugated phenyl 10-methyl-9-acridiniumcarboxalates, in our hands, are unstable due to hydrolysis above pH 4.0 (-20°C to 40°C), losing greater than 10% of their activity within three days. Although acridinium esters are stable below pH 4.0, conjugate antibodies are often not stable in this pH range.

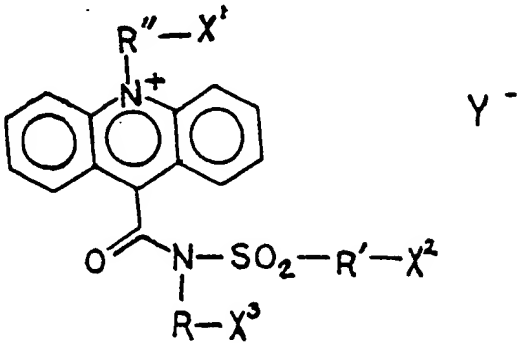
In Tseng et al., supra, bis-N-alkyl-N-trifluoromethyl sulfonyl oxalamides are indicated to be more stable than the corresponding aryl esters and are also indicated to be as efficient. The nucleofugacity of the phenol and the trifluoromethyl sulfonamide are indicated to be comparable, i.e. it is indicated that each has a pK_a of about 7. Gill, supra , "look forward" to the development of a particular sulfonyl oxalamide as an example of an oxalate with "higher" quantum efficiency.

Brief Description of the Drawings

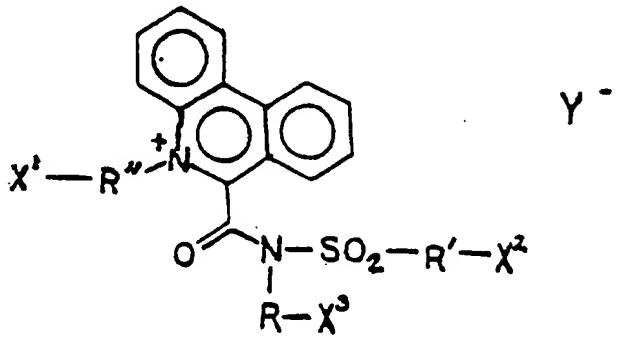
The Figure illustrates the synthesis of a 10-alkyl-N-sulfonyl-9-acridinium carboxamide according to the present invention.

Summary of the Invention

The present invention provides chemiluminescent compounds identified by the formula



and isomers thereof including isomers identified by the formula



wherein R, R', R", X^1, X^2, and X^3 are substituents which do not interfere with effective chemiluminescence, with the proviso that R-X^3, R'-X^2 and R"-X^1 may be independently hydrogen. More specifically, R, R' and R" may be spacer arms and X^1, X^2 and X^3 may be independently members of the group consisting of hydrogen, carboxy, carboalkoxyl, carboxamido, carboaryloxy, cyano, carboximido, isocyanato, isothiocyanato, sulfo, sulfonyl halide, carbonyl halide, N-succinimidyloxycarbonyl and N-maleimide groups. Y is an appropriate counterion and may be selected from the group consisting of sulfate, alkylsulfate, halosulfate, haloborate, haloacetate, halophosphate, phosphate and halide.

R, R', and R" may independently include a member selected from the group consisting of alkyl, alkylene, aryl, substituted alkyl, substituted alkylene and substituted aryl groups, such that one or more hydrogens of said member is replaced by an alkyl, aryl, alkylene, substituted alkyl, substituted alkylene, substituted aryl, alkoxy, aryloxy, halo, amino, protected amino, substituted amino hydroxy, protected hydroxy, oxo, thio, imino, mercapto or substituted mercapto group; or such that one or more carbon atoms of the member is replaced by a heteroatom. The heteroatom may be selected from the group consisting of nitrogen, phosphorus, sulfur and oxygen.

R, R', and R" independently may also be spacer arms of the formula

$-(CH_2)_n-$

where n = 0 - 50. Specifically, R" may be $-CH_2-$ and X^1 may be -H.

The currently most preferred compounds according to the present invention for use in chemiluminescent immunoassays are 10-methyl-N-[2-carboxyethyl]-N-tosyl-9-acridinium carboxyamide, 10-(3-sulfopropyl)-N-(2-carboxyethyl)-N-tosyl-9-acridinium carboxamide and 1-(3-sulfopropyl)-N-(3-sulfopropyl)-N-tosyl-9-acridinium carboxamide.

A method, according to the present invention, for preparation of a chemiluminescent compound includes th steps of contacting an amine identified by the formula

X^3-R-NH_2

with a sulfonylhalide identified by the formula

W-SO_2-R'-X^2

in an inert solvent in the presence of base to form a sulfonamide anion and metal ion identified by the formulas

$$X^3-R-\overset{-}{N}-SO_2-R'-X^2 \quad M^+ \quad ;$$

and acrylating with an activated 9-acridinecarboxylate compound according to the present invention, wherein W is selected from the group; consisting of chloro and fluoro groups, wherein M is selected from the group consisting of Li, Na and K, wherein the activating group is selected from the group consisting of halo, imidazolo, N-hydroxysuccinimidyl and azido groups and wherein all other symbols are as defined above.

A conjugate according to the present invention may be formed by covalently coupling an antibody, a hapten, an antigen or a polynucleotide (e.g., DNA or RNA) to a chemiluminescent compound according to the present invention, and a method for performing a chemiluminescent assay comprises the step of exposing a sample to be tested to the conjugate in order to detect the presence of a substance specifically reactive with the conjugate, e.g., a specifc antigen, a specific antibody or a complementary polynucleotide (i.e., a polynucleotide which forms sequence-specific hydrogen bonds with the polynucleotide conjugate according to the present inveniton).

Detailed Description

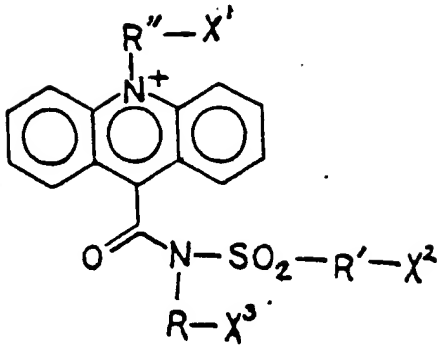
The problem of acridinium aryl ester instability is approached in the present invention by changing the leaving group from a phenolate to a sulfonamide anion. While both leaving groups have a pK_a of about 10, the acridinium sulfonylamide has the additional stabilization associated with amide bonds. This is reflected in a comparison in the infrared of the carbonyl stretching frequency of the aryl ester (1730 cm^{-1}) with that of the sulfonylamide (1680 cm^{-1}).

A class of acridinium salts, 10-alkyl N-alkyl (aryl) sulfonyl-N-alkyl(aryl) 9-acridinium carboxamide salts, was prepared according to the general scheme illustrated in the Figure. In the Figure, R, R' and R" are substitutents which may function as spacer arms, solubility modifiers and/or reactivity modifiers but which do not interfere with the chemiluminescent reaction. ("Interfere" is defined herein to mean "prevent the production of effective chemiluminescence", i.e., prevent production of chemiluminescence to the extent that the compound is not useful for the intended application.) Also in the Figure, X^1, X^2, X^3 are substituents which may function as solubility enhancers and/or as reactive groups for linkage to an analyte or as groups which may be readily converted to such reactive or linker groups by means well known to those skilled in the art. Y is a counterion in the Figure.

Salts produced according to the scheme of the Figure have generated light upon oxidation with alkaline hydrogen peroxide. The compounds were made from readily available amines (X^3-RNH_2) and sulfonyl chlorides (X^2-$R'SO_2Cl$). When acrylated with 9-chlorocarbonyl acridine, the intermediate sulfonamide (X^3-RNH-SO_2R^1-X_2) gave a new class of acridine compounds, which on alkylation gave the acridinium salts. Similarly, substitution of a 6-chlorocarbonyl phenanthridine for the acridine in this scheme gives rise to a new class of phenanthridinium salts. These acridinium and phenanthridinium salts are useful for chemiluminescent labeling of proteins, nucleic acids and small molecules used in diagnostic testing.

Several acridinium sulfonylamides were prepared which have specific activity and stability suitable for use in diagnostic testing, particularly in CLIA. The synthesis of these compounds allows for the introduction of a variety of functional groups (X^1, X^2, X^3) which may be used in antibody labeling. In addition, the kinetics of the chemiluminescent reaction may be controlled by the choice of the substitutents (R, R') on the sulfonamide leaving group.

The compounds were evaluated for their efficiency by diluting 20 µl of a 10^{-9} M solution of the compound with 300 µl of 0.1N HCL, then adding 150 µl of 0.03% H_2O_2 in 0.2 N NaOH to trigger the chemiluminescence. Chemiluminescence was measured on a photon-counting luminometer. The light output was recorded as total photon counts, from which the efficiency of each compound was calculated as counts/mole. These are relative numbers, since the efficiency of the photon counting was instrument-dependent. Direct comparisons of compounds were carried out on the same instrument. The results are presented in Table I which the structures may be identified by the formula



wherein R"-X^1 is CH_3, and R'-X^2 and R-X^3 are as indicated in Table 1, chemiluminscent output is abbreviated "CTS/MOLE," the time required for total light output is abbreviated "INT. TIME" and the time required to reach peak light output is abbreviated "PEAK CTS.".

TABLE 1

$R-X^3$	$R'-X^2$	CTS/MOLE ($X\ 10^{-18}$)	INT. TIME	PEAK CTS (SEC)
CF_3	C_6H_5	12	1	0.22
$o-NO_2C_6H_4$	C_6H_5	10	2	0.23
$p-Br-C_6H_4$	C_6H_5	9	2	0.24
CF_3	$i-C_3H_7$	15	2	0.25
$p-NO_2-C_6H_4$	$n-C_4H_9$	8	2	0.25
$o-NO_2-C_6H_4$	$i-C_3H_7$	11	2	0.25
$p-CH_3C_6H_4$	C_6H_5	9	2	0.27
$o-NO_2C_6H_4$	$n-C_4H_9$	6	2	0.29
$2,4-di-NO_2C_6H_3$	$n-C_4-H_9$	5	2	0.32
$p-BrC_6H_4$	$n-C_4H_9$	7	3	0.44
$p-BrC_6H_4$	$i-C_3H_7$	12	6	0.44
$p-CH_3C_6H_4$	$n-C_4H_9$	5	6	0.98
$p-CH_3C_6H_4$	$i-C_3H_7$	8.3	10	0.96
$2,4,6-(C_3H_7)_3C_6H_2$	$n-C_4H_9$	14	20	4.08
$2,4,6-(CH_3)_3C_6H_2$	$n-C_4H_9$	5	50	11.6
CF_3	$C_6H_4CHCO_2Bn$	4	2	--

All of the tested compounds were efficient (5-20 x 10^{18} counts/mole). The specific activity was insensitive to the nature of the R and R' groups at locations indicated above; however, the time required to reach peak light output and the time required for total light output differed by a factor of 50 between the fastest and slowest compounds. Electron withdrawing groups in R and R' increased the reaction rate while bulkyl, electfon-donating groups decreased the reaction rate. Although chemiluminscent compounds according to the present invention which have a chemiluminescent lifetime of 2-10 seconds are preferred for immunoassays, compounds having shorter lifetime may be useful as a source of intense, pulsed light, and compounds having a longer lifetime may be useful as "cold light" sources.

The stability of compounds prepared according to the present invention was assessed in several ways. First, the compounds were diluted to sub-nanomolar solutions in aqueous buffer at pH 5-7. The solutions

were incubated at room temperature and at 45°C, while the decrease in chemiluminescence was monitored over time. This provided qualitative results whereby the relative stability of the compounds was determined. Anomalous results due to non-specific adsorption of the compounds on the incubation container were minimized by the addition of detergents, protein, and the like. Unambiguous, quantitative results were obtained by monitoring millimolar solutions of the compounds by reverse phase high performance liquid chromatography ("HPLC"). The stability of these compounds was affected by R' and R' in the same way as were the kinetics of the chemiluminescence reaction, i.e. electron withdrawing groups destabilized and bulky electron donating groups stabilized the compounds.

Although other techniques may be employed to label antibodies, the NHS activation method is presently preferred. Other materials which function well according to the present invention include polyclonal antibodies, monoclonal antibodies, Fab antibody fragments, all of which are hereinafter included in the general term "antibody," haptens, antigens, nucleic acid probes, and non-antibody binding proteins capable of binding complementary small molecular weight analytes (for example, folate binding protein, which binds folic acid, and intrinsic factor, which binds Vitamin B_{12}). Antibody conjugates retain more than 80% chemiluminescence after being heated at 45°C for four weeks.

A solid phase sandwich immunoassay system for assaying hepatitis B surface antigen ("HBsAg") (Abbott Laboratories, Abbott Park, Illinois) was employed to compare CLIA according to the present invention with RIA. The type of antibody-coated bead, diluent, incubation conditions, washing condition and antibody preparation were the same except that the antibody was labeled with ^{125}I by the chloramine T method for RIA and labeled with NHS-activated N-sulfonyl-9-acridinium carboxamide for CLIA.

A solid phase sandwich immunoassay for human thyroid stimulating hormone (hTSH) was used to compare CLIA with EI (Abbott Laboratories, Abbott Park, Illinois). The EIA employed a horseradish peroxidase ("HRPO")-labelled antibody while the CLIA used an NHS-activated N-sulfonyl-9-acridinium carboxamide.

The present invention is more specifically described in the following examples. In Example 1, the preparation of sulfonamides which are useful in constructing compounds according to the present invention is set forth. Example 2 includes a description of the preparation of N-sulfonyl-9-acridinecarboxamides according to the present invention. In Example 3, the preparation of 10-methyl N-sulfonyl-acridinium carboxamides is described. Examples 4-6 contain descriptions of syntheses of p-toluenesulfonyl (tosyl) compounds according to the present invention. In Example 7, the preparation of acridinecarboxamides is illustrated.

Example 8-10 contain methods for synthesis of some acridinium carboxamides and products thereof according to the present invention. In Example 11, an evaluation of the chemiluminescence of N-sulfonylacridinium carboxamide compounds according to the present invention is provided. Example 12 includes a report of a stability test of an acridinium carboxamide according to the present invention. In Example 13, the temperature and pH stability of two acridinium carboxamides according to the present invention is compared to the temperature and pH stability of an acridiniumcarboxylate. Example 14 is a description of a method for conjugating an antibody, specifically an immunoglobin G ("IgG") antibody, with a compound according to the present invention. The results of a heat stability study of a conjugate according to Example 14 are presented in Example 15. Example 16 includes a description of the preparation of anti-HBsAg acridinium-labeled conjugate as well as a comparison of the sensitivity observed in CLIA and RIA assays employing those conjugates. In Example 18, the synthesis of a phenanthridinium compound according to the present invention is described. Example 17 describes an anti-hTSH acridinium-labeled conjugate along with a comparison to an EIA system.

Example 1

General Method for Preparation Of Sulfonamides

Amine starting materials for compounds 1-13 and 17-21 are available from Aldrich Chemical Co., Milwaukee, Wisconsin. For compounds 14-16 and 22-25, the appropriate aminocarboxylic acid (as obtained from Aldrich Chemical Co., Milwaukee, Wisconsin) was esterified according to standard, published procedures to provide the starting materials.

In order to prepare a sulfonamide according to the present invention, the corresponding amine (200 mole percent) was dissolved in anhydrous methylene chloride, and was treated dropwise at 0°C with a soluti n (100 mole percent) of the sulfonyl chloride or anhydride. The solution was poured into anhydrous ether (5 volumes), washed with 1.4 M H_3PO_4 (25 ml) and then brine (25 ml), and dried over $MgSO_4$. After

filtering and evaporating, crude sulfonamid s w re crystallized from an appropriate solv nt.

The following sulfonamides were prepared in this manner. In the description accompanying the name of each compound, the abbreviation "MS" identifies peaks, such as the base peak ("M ") in the mass spectrum at a location (i.e., at an m/e) specified by the symbol "@". A melting point ("M_p") or an indication that th material is a liquid at room temperature (.g. "oil") or decomposes before melting ("decomp.") may be provided. Each compound is identified by a "compound number" (1-25 in this Example) followed by an "identifying number" (e.g. 13513-227) and a chemical name.

1. 13513-227 N-Phenyl-p-toluenesulfonamide
MS M^+ @ 247
M_p 100-102°C

2. 13513-228 N-Phenyl-p-bromobenzenesulfonamide
MS M^+ @ 311
M_p 115-117°C

3. 13513-229 N-Phenyl-o-nitrobenzenesulfonamide
MS M^+ @ 278
M_p 112-113°C

4. 13513-231 N-Phenyl-p-nitrobenzenesulfonamide
MS M^+ @ 278
M_p 168-170°C

5. 13513-232 N-Phenyl-2,4-dinitrobenzene-sulfonamide
MS M^+ @ 323
M_p 110-113°C

6. 13513-233 N-Phenyl-trifluoromethane-sulfonamide
MS M^+ @ 225
M_p 65-67°C

7. 13514-001 N-Isopropyl-p-toluenesulfonamide
MS M^+ @ 213
M_p 50-51°C

8. 13514-002 N-Isopropyl-p-bromobenzenesulfonamide
MS M^+ @ 277
M_p 95-96°C

9. 13514-003 N-Isopropyl-o-nitrobenzenesulfonamide
MS M^+ @ 244
M_p 119-120°C

10. 13514-004 N-Isopropyl-trifluoromethanesulfonamide
MS (M - 1) @ 190
oil

11. 13514-006 N-Isopropyl-p-nitrobenzenesulfonamide
MS M^+ @ 244
M_p 113-114°C

12. 13514-025 N-Butyl-2,4,6-trimethylbenzenesulfonamide
MS M^+ @ 255
M_p 45°C

13. 13514-026 N-Butyl-2,4,6,-trisopropylbenzenesulfonamide
MS M^+ @ 339
M_p 104°C

14. 13514-032 Benzyl 6-(N-tosylamino)-hexanoate
MS M^+ @ 375
oil

15. 13514-057 t-Butyl N-tosyl-β-alanine
MS M^+ @ 242 (M - 57)
oil

16. 13514-058 Benzyl 5-(N-tosylamino)-pentanoate
MS M^+ @ 361
oil

17. 13513-170 N-Butyl-p-toluenesulfonamide,
MS M^+ @ 227
M_p 42-44°C

18. 13513-173 N-Butyl-p-bromobenzenesulfonamide,
MS M^+ @ 241
M_p 53-54°C

19. 13513-172 N-Butyl-o-nitrobenzenesulfonamide,
MS M^+ @ 258
M_p 58-60°C

20. 13513-174 N-Butyl-p-nitrobenzenesulfonamide
MS M^+ @ 258
M_p 80-81°C

21. 13513-213 N-Butyl-2,4-dinitrobenzene sulfonamide,
MS M^+ @ 304
M_p 60-62°C

22. 13513-085 Benzyl 6-(N-trifluoromethyl-sulfonylamino)-hexanoate
oil

23. 13513-083 Benzyl N-(trifluoromethylsulfonyl)-4-(carboxymethyl) aniline

24. 14973-1A Benzyl N-(5-carboxypentyl)-p-bromobenzenesulfonamide
MS M^+ @ 439
M_p 52-56°C

25. 14973-37A Benzyl N-(5-carboxypentyl)-p-nitrobenzenesulfonamide
MS M^+ @ 406
M_p 86-88°C

Example 2

Preparation of N-sulfonyl-9-acridinecarboxamides

Freshly sublimed potassium tert-butoxide (200 mole percent) and tri-n-butylbenzylammonium bromide (1 mole percent) were suspended in toluene under nitrogen. A selected sulfonamide (200 mole percent) was added, the mixture was stirred for 10-30 minutes before evaporating to dryness and the dried material resuspended in the solvent. [Alternatively, the phase transfer catalyst may be omitted and an appropriate anion may be generated in tetrahydrofuran.] After the addition of 9-chlorocarbonylacridine hydrochloride (200 mole percent), the reaction mixture was stirred for 3 to 14 hours at room temperature until no further change was noted by thin-layer chromatography ("TLC"). The reaction solution was diluted with ethyl ether (10 volumes) and washed with brine (25 ml). After drying over $MgSO_4$, filtering and evaporating, the crude product was chromatographed (on a Chromatotron™ chromatograph [available from Harrison Research, Palo Alto, California] using a 2 mm silica rotor and employing an ethylacetate/hexane gradient). The fractions containing the product were collected, evaporated and crystallized from ether/heptane (i.e., the fractions were dissolved in ether followed by the addition of heptane until the mixture became cloudy).

The following compounds were prepared from starting materials as indicated in brackets wherein starting materials prepared herein are identified by the number associated with them in Example 1 or in this example, and wherein a commercial source is provided in brackets for each identified starting material not synthesized herein. All other notations are explained in Example 1.

26. 13513-234 N-Phenyl-N-p-toluenesulfonyl-9-acridinecarboxamide
[compound 1]
MS M^+ @ 452
M_p 200°C

27. 13513-236 N-Phenyl-N-p-bromobenzene-sulfonyl 9-acridinecarboxamide
[compound 2]
MS M^+ @ 516
M_p 218-219°C

28. 13513-240 N-Phenyl-N-o-nitrobenzene-sulfonyl 9-acridinecarboxamide
[compound 3]
MS M^+ @ 483
M_p 197-200°C

29. 13513-242 N-Phenyl-N-p-nitrobenzene-sulfonyl-9-acridinecarboxamide
[compound 4]
MS M^+ @ 483

30. 13513-243 N-Phenyl-N-trifluoromethane-sulfonyl-9-acridinecarboxamide
[compound 6]
MS M^+ @ 430
M_p 162°C

31. 13514-007 N-Isopropyl-N-p-toluene-sulfonyl-9-acridinecarboxamide
[compound 7]
MS M^+ @ 418
M_p 163-164°C

32. 13514-009 N-Isopropropyl-N-p-bromobenzenesulfonyl-9-acridinecarboxamide
[compound 8]
MS M^+ @ 482
M_p 205°C

33. 13514-012 N-Isopropyl-N-o-nitrobenzene-sulfonyl-9-acridinecarboxamide
[compound 9]
MS M^+ @ 449
M_p 215°C

34. 13514-001 N-Isopropyl-N-trifluoromethane sulfonyl-9-acridinecarboxamide
[compound 10]
MS M^+ @ 396

35. 13514-028 N-Butyl-N-2,4,6,-trimethyl-benzenesulfonyl-9-acridine-carboxamide
[compound 12]
MS M^+ @ 460
M_p 88-90°C

36. 13514-031 N-Butyl-2,4,6-triisopropylbenzene-sulfonyl-9-acridinecarboxamide
[compound 13]
MS M^+ @ 544

37. 13514-042 Benzyl N-tosyl-N-(5-carboxypentyl)-9-acridinecarboxamide
[compound 14]
MS M^+ @ 550
oil

38. 13514-062 Benzyl N-tosyl-N-(4-carboxybutyl)-9-acridinecarboxamide
[compound 16]
MS M^+ @566

39. 13514-069 t-Butyl N-tosyl-N-(2-carboxyethyl)-9-acridinecarboxamide
[compound 15]
MS M^+ 504
M_p 157-158°C

40. 13513-186 N-Butyl-N-p-toluenesulfonyl-9-acridinecarboxamide
[compound 17]
MS M^+ @ 432
M_p 122-123°C

41. 13513-191 N-Butyl-N-o-nitrophenylsulfonyl-9-acridinecarboxamide
[compound 19]
MS M^+ @ 463
M_p 170°C

42. 13513-195 N-Butyl-N-p-nitrophenylsulfonyl-9-acridinecarboxamide
[compound 20]
MS M^+ 463
M_p 210°C

43. 13513-218 N-Butyl-N-(2,4-dinitrophenylsulfonyl)-9-acridinecarboxamide
[compound 21]
MS M^+ @ 508
M_p 95°C

44. 14973-9C	Benzyl N-(5-carboxypentyl)-N-p-bromobenzenesulfonyl-9-acridinecarboxamide [compound 24] MS (M + H) @ 645
45. 14973-40C	Benzyl N-(5-carboxypentyl)-N-p-nitrobenzenesulfonyl-9-acridinecarboxamide [compound 25] MS (M + H) @ 645
46. 14973-88A	N-p-Toluenesulfonyl-9-acridinecarboxamide [p-toluene sulfonamide (Aldrich)] M_p 276°C
47. 14973-21C	N-Allyl-N-p-toluenesulfonyl-9-acridinecarboxamide [compound 46] M_p 136-138°C
48. 13513-202	N-Butyl-N-p-bromobenzenesulfonyl-9-acridinecarboxamide MS M^+ @ 496/498 M_p 148-149°C

Example 3

Preparation of 10-Methyl N-sulfonylacridinium carboxamides

Methylation of N-sulfonylacridine carboxamides was performed according to the following procedure. Each acridine sulfonylamide was dissolve in anydrous methylene chloride. Anhydrous Na_2CO_3 (5 X weight of the sulfonamide) was added followed by methyl triflate (20 X weight of the sulfonimide). The suspension was stirred under nitrogen for 14-48 hours at room temperature to 40°C. The reaction was monitored by TLC (reverse phase). The product was obtained after filtration and evaporation of the solvent and of excess methyl triflate. Purification was achieved by triturating the solid residue with hot benzene or by reverse phase HPLC.

The following compounds were prepared, and they are described according to the numerals, symbols and abbreviations which are explained in Example 1 or in Example 2.

49.	13513-246	10-Methyl-N-phenyl-N-p-toluenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate [compound 26] MS M^+ @ 467 M_p 210-24°C (decomp.)
50.	13513-247	10-Methyl-N-phenyl-N-p-bromobenzenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate [compound 27] MS M^+ @ 531, 533 M_p 240°C (decomp.)
51.	13513-248	10-Methyl-N-phenyl-o-nitro-benzenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate [compound 28] MS M^+ @ 490 M_p 248-50°C (decomp.)

52.	13513-249	10-Methyl-N-phenyl-N-trifluoromethanesufonyl-9-acridinium carboxamide trifluoromethanesulfonate [compound 30] MS M^+ @ 445
53.	13513-250	10-Methyl-N-phenyl-p-nitrobenzenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate [compound 29] MS M^+ @ 484
54.	13514-013	10-Methyl-N-isopropyl-N-p-toluenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate [compound 31] MS M^+ @ 433 M_p 214°C
55.	13514-014	10-Methyl-N-isopropyl-N-p-bromobenzenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate [compound 32] MS M^+ @ 497/499 M_p 200°C (decomp)

56. 13514-018 10-Methyl-N-isopropyl-N-o-nitrobenzenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate
[compound 33]
MS M^+ @ 464

57. 13514-021 10-Methyl-N-isopropyl-N-trifluoromethanesulfonyl-9-acridinium carboxamide trifluormethanesulfonate
[compound 34]
MS M^+ @ 411

58. 13514-037 10-Methyl-N-butyl-N-(2,4,6-trimethylbenzenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate
[compound 35]
MS M^+ @ 475
M_p 227°C (decomp.)

59. 13514-038 10-Methyl-N-butyl-N-(2,4,6 triisopropylbenzenesulfonyl-9--acridinium carboxamide trifluoromethanesulfonate
[compound 36]
MS M^+ @ 559
M_p 231°C (decomp.)

60. 13514-044 Benzyl 10-methyl-N-tosyl-N-(5-carboxypentyl)-9-acridinium carboxamide trifluoromethanesulfonate
[compound 37]

61. 13514-079	t-Butyl 10-methyl-N-tosyl-N-(2-carboxyethyl)-9-acridinium carboxamide trifluoromethanesulfonate [compound 39] MS M^+ @ 519 M_p 207°C (decomp.)
62.. 13513-211	10-Methyl-N-butyl-N-p-toluenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate. [compound 40] MS M^+ @ 447
63. 13513-212	10-Methyl-N-butyl-N-p-bromobenzenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate [compound 48] MS M^+ @ 511 M_p 126°C
64. 13513-215	10-Methyl-N-butyl-N-o-nitrophenylsulfonyl-9-acridinium carboxamide trifluoromethanesulfonate [compound 41] MS M^+ @ 478 M_p 232-234°C

65. 13513-216 10-Methyl-N-butyl-N-p-nitrophenysulfonyl-9-acridinium carboxamide trifluoromethanesulfonate
[compound 42]
MS M^+ @ 478
M_p 201°C

66. 13513-230 10-Methyl-N-butyl-N-(2-4 dinitrophenylsulfonyl)-9-acridinium carboxamide trifluoromethanesulfonate
[compound 43]
MS M^+ @ 523
M_p 215-220°C

67. 14973-31B 10-Methyl-N-allyl-N-p-toluenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate
[compound 47]
MS M + 2 @ 433

68. 14973-47A Benzyl 10-methyl-N-(5-carboxypentyl)-N-p-nitrobenzenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate
[compound 45]
MS M^+ @ 626
M_p 139-141°C

69. 14973-90A 10-Methyl-N-methyl-N-p-toluenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate
[compound 46]
MS M^+ @ 405

70. 14973-25A Benzyl 10-methyl-N-(5-carboxypentyl)-N-(o-bromobenzenesulfonyl)-9-acridinium carboxamide
[compound 44]

Example 4

Synthesis of 10-methyl-N-tosyl-N-(6-hexanoyl-N-hydroxysuccinimido)-9-acridinium carboxamide trifluoromethanesulfonate

Compound 37 (450 mg, 0.78 mmoles) was treated with 6 ml of 31% HBr in acetic acid at 50°C for 2 hours under N_2. The solution was poured into 30 ml of water and cooled. Carboxylic acid compound 71, 13514-045 [N-tosyl-N-(5-carboxypentyl)-9-acridinecarboxamide] was separated by filtration.

Compound 71 (100 mg., 0.2 mmol) was dissolved in dry methylene chloride (5 ml) and treated with N-hydroxysuccinimide (23 mg, 0.2 mmol) and dicyclohexylcarbodiimide (41 mg) under N_2 for 12 hours. After reacting, the solution was filtered and then evaporated to dryness to yield an active ester, compound 72, 13514-952 [N-tosyl-N-(6-hexanoyl-N-hydroxysuccinimido)-9-acridinecarboxamide].

Compound 72 was methylated as in Example 3 to give compound 73. Compounds 71, 72 and 73 are described below using the numerals, symbols and abbreviations which are explained in Example 1.

explained in Example 1.

71. 13514-045 N-Tosyl-N-(5-carboxypentyl)-9-acridinecarboxamide
[compound 37]
MS M^+ @ 240
M_p 150-152°C

72. 13514-052 N-Tosyl-N-(6-hexanoyl-N-hydroxysuccinimido)-9-acridinecarboxamide
[compound 71]
MS M^+ @ 588

73. 13514-054 10-Methyl-N-tosyl-N-(6-hexanoyl-N-hydroxysuccinimido)-9-acridinumcarboxamide trifluoromethanesulfonate
[compound 72]

Example 5

Synthesis of 10-Methyl-N-tosyl-N-(5-pentanoyl-N-hydroxysuccininimido)-9-acridinium carboxamide trifluoromethanesulfonate

Compound 38, 13514-062, was treated as in Example 4 and yielded compound 74, 13514-065 [N-tosyl-N(4-carboxybutyl)-9-acridinecarboxamide].

Compound 74 was coupled to N-hydroxysuccinimide, as in Example 4, to give compound 75, 13514-067, N-tosyl-N-(5-pentanoyl-N-hydroxysuccinimido)-9-acridinecarboxamide. This compound was methylated as in Example 3 to give compound 76, 13514-78 [10-methyl N-tosyl-N-(5-pentanoyl-N-hydroxysuccinimide)-9-acridinium carboxamide trifluoromethanesulfonate].

Compounds 74, 75 and 76 are described using the numerals, symbols and abbreviations which are explained in Example 1.

74. 13514-065 N-Tosyl-N-(4-carboxybutyl)-9-acridinecarboxamide
MS M^+ @ 476
M_p 152-155°C

75. 13514-067 N-Tosyl-(5-pentanoyl N-hydroxy succinimido)-9-acridinecarboxamide
[compound 74]
MS M^+ @ 573

76. 13514-078 10-Methyl-N-tosyl-N-(5-pentanoyl-N-hydroxy-succinimido)-9-acridinium carboxamide trifluoromethanesulfonate
[compound 75]

Example 6

Synthesis of 10-methyl-N-tosyl-N-(2-carboxyethyl-9-acridinium carboxamide trifluoromethanesulfonate

Compound 61, 13514-079 (50 mg, 0.072 mmol) was dissolved in 2 ml of trifluoroacetic acid ["TFA"] at 0°C under N_2. After stirring for 15 minutes, the TFA was evaporated and the residue was recrystallized from methanol/ether (i.e., the residue was dissolved in methanol, adding ether until cloudy). Alternatively, compound 61, was refluxed in 1 N HCl for 3 hours. The aqueous solution was evaporated to dryness to leave a residue, and the residue was purified by preparative reverse phase HPLC. Compound 77, 13514-081 [10-methyl N-tosyl-N-(2-carboxyethyl)-9-acridinium carboxamide] resulted from either approach. Compound 77 is described using the numerals, symbols and abbreviations which are explained in Example 1.

77. 13514-081 10-Methyl-N-tosyl-N-(2-carboxyethyl)-9-acridinium carboxamide trifluoromethanesulfonate
[compound 61]
MS (M + 14) @ 477; M^+ @ 463
M_p 227°C (decomp.)

Example 7

Preparation of Acridinecarboxamides

An amine (110 mole percent) and triethylamine (220 mole percent) were dissolved in methylene chloride. One hundred mole percent of 9-chlorocarbonyl acridine was added dropwise as a solution in methylene chloride. The r action was stirred under N_2 for 3 hours. The solution was filtered through silica gel and the filtrate was evaporated to leave a residue. The residue was then recrystallized from an appropriate solvent (isopropyl ether for compound 78 and ethyl ether for compound 79).

The following amides were prepared, and are described using the numerals, symbols and abbreviations which are explained in Example 1.

78. 14973-15A N-Allyl-9-acridinecarboxamide
[Allyl amine (Aldrich)]
MS M^+ @ 262
M_p 192°C

79. 14973-6A Benzyl N-(5-carboxypentyl)-9-acridinecarboxamide
[6-Amino caproic acid (Aldrich)]
MS M^+ @ 458
M_p 86°C

Example 8

Synthesis of Acridinium carboxamides

An ester (either compound 44 or compound 68) was added to a 1 N HCl solution and refluxed for 3-4 hours. Upon cooling, the suspension was either filtered and the product collected, or the suspension was extracted with a chloroform:isopropanol (3:2) mixture, which provided the desired product (compound 80 or 81, respectively) on evaporation. Compounds 80 and 81 are described using the numerals, symbols and abbreviations which are explained in Example 1.

80. 14379-27A 10-Methyl-N-(5-carboxypentyl)-N-p-bromobenzenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate
[compound 44]
MS M^+ @ 569, 571
M_p 148-150°C

81. 14973-51A 10-Methyl-N-(5-carboxypentyl)-N-p-nitrobenzenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate
[compound 68]
MS M^+ @ 536

Example 9

Synthesis of 10-(3-sulfopropyl)-N-tosyl-N-(2-carboxyethyl)-9-acridinium carboxamide

Propane sultone (260 mole percent) was heated with t-butyl N-tosyl-N-(2-carboxyethyl)-9-acridinecarboxamide (compound 39, 13514-069) at 110 -120°C for 2 hours. After cooling, the solid mass was taken up in methanol and filtered. The filtrate was evaporated to dryness and the residue triturated with benzene to remove un-quaternized material.

The crude product compound was treated with trifluoracetic acid at 0°C then allowed to warm to 25°C over a period of 15 minutes. The residue obtained upon evapoation was purified chromatographically on preparative thick-layer chromatography plates (C-18 PLKC 18F, 20 x 20 cm, 1000M, as available from Whatman, Clifton, New Jersey), eluted with 70 parts methanol/30 parts 0.5% aqueous acetic acid, and further purified by ion exchange on Cellex-DTM resin [BioRad Laboratories, Richmond, California] using 8% formic acid to elute the product, compound 82, which is described below using the numerals, symbols and abbreviations which are explained in Example 1.

82. 14496-243 10-(3-sulfopropyl)-N-tosyl-N-(2-carboxyethyl)-9-acridinium carboxamide
[compound 39]
MS M^+ @ 572

Example 10

Synthesis of 10-(3-sulfopropyl)-N-tosyl-N-(3-sulfopropyl)-9-acridinium carboxamide

Fifty milligrams of N-tosyl-9-acridinecarboxamide (compound 46, 14973-88A) were heated at 140-150°C under argon in a sealed tube with 500 mg of propane sultone for 3 hours. After cooling, excess propane sultone was removed by trituration with benzene (5 ml X 3). The crude product was purified by anion exchange chromatography using BioRad AG-1-X4 formate form [BioRad Laboratory, Richmond, California], eluted with a gradient of aqueous formic acid. The product, compound 83, is described below using the numerals, symbols and abbreviations explained in Example 1.

83. 30253-020 10-(3-Sulfopropyl)-N-tosyl-N-(3-sulfopropyl)-9-acridinium carboxamide.
[compound 46]
MS M + H @ 621.

Example 11

Evaluation of N-sulfonylacridinium carboxamide Chemiluminescence

Acridinium compounds to be tested for chemiluminescence were dissolved in dimethyl formamide ("DMF") and then diluted with 0.05 M sodium citrate (pH 5.0) of 0.05 M sodium phosphate (pH 7.0) buffer to give solutions of about 3×10^{9} M. Twenty microliters of each buffered solution was diluted with 300 µl of 0.1 N HCl and chemiluminscence was triggered with 150 µl of 0.03% H_2O_2 in 0.2 N NaOH.

The light generated was recorded on a photon counter luminometer over a 10 second interval except where a longer interval is indicated in Table 1. The specific activity of each compound is provided in the form of counts/moles in Table 1.

TABLE 2

Compound No.	Identifying No.	Counts/Mole
49	13513-246	9.4×10^{18}
50	13513-247	9×10^{18}
51	13513-248	1×10^{19}
50	13513-249	1.2×10^{19}
53	13513-250	1×10^{19}
54	13514-013	8.3×10^{18}
55	13514-014	1.25×10^{19}
56	13514-018	1.1×10^{19}
57	13514-021	1.5×10^{19}
58	13514-037	5.2×10^{18} (50 secs)
59	13514-038	1.4×10^{19} (20 secs)
62	13513-211	5×10^{18}
63	13513-212	7×10^{18}
64	13513-215	6.1×10^{18}
65	13513-216	8×10^{18}
66	13513-230	5×10^{18}

Example 12

Stability Test of Compound 62 (13513-211)

Compound 62 (2 mg) was dissolved in 1 ml of methanol. Fifty microliters of this solution were added to each of the following buffers:

1) 500 microliters of 0.05 M sodium phosphate, pH 5.0
2) 500 microliters of 0.05 M sodium phosphate, pH 5.5
3) 500 microliters of 0.05 M sodium phosphate, pH 6.0

4) 500 microliters of 0.05 M sodium phosphate, pH 6.5

5) 500 microliters of 0.05 M sodium phosphate, pH 7.0. Each solution was analyzed on a Perkin-Elmer Series 4 HPLC using a reverse phase column (C-18 μ Bondapak, 3.9 mm x 30 cm, available from Waters Associates, Milford, Massachusetts). The elution was done with 75% methanol and 25% 5 mM pentanesulfonic acid in 1% aqueous acetic acid at a flow rate of 1 ml/min. The effluent was monitored at 254 nm.

After 4 weeks at room temperature, the solutions at pH 5.0, pH 5.5 and pH 6.0 showed no sign of decomposition, while at pH 6.5 and at pH 7.0, 20% and 70% decomposition were seen, respectively.

Example 13

Comparison of Temperature and pH Stabilities of Acridinium Compounds in Buffer at pH 7.2

Three different acridinium compounds, compound 62, 13513-211, a compound identified by the number 13514-020 [4-(carbobenzyloxymethyl)-phenyl-10-methyl-9-acridinium carboxylate trifluoromethanesulfonate] as prepared as in Weeks, et al., Clin. Chem. , 29, 1474-79 (1983), and compound 83, 30253-020, were compared for temperature and pH stability. The comparison was carried out in methanol or water at a concentration of 1.0 mg/ml (which is approximately equivalent to 1.6×10^{-3} M). Each of the samples was diluted 1:100 in an acid solution containing one part of 0.1 N HCl plus one part phosphate-buffered saline ("PBS") pH 6.8 with 0.01% Tween 20® (available from Sigma Chemical Company, St. Louis, Missouri). The final pH of the diluent solution were about 1.5. The molarity of each of these solutions was 1.6×10^{-5} M.

Each of the solutions was scanned to record a UV-visible absorption spectrum in order to determine molar extinction coefficients and in order to detect any appreciable differences in the absorbance spectra. The UV-visible absorption spectra of these acridinium compounds have the characteristics presented in Table 2.

TABLE 3

Compound No.	Identifying No.	Wavelength	Observed Absorbance
62	13513-211	263nm	1.40
		369nm	0.286
83	30253-020	263.5nm	1.42
		370nm	0.304
	13514-020	262nm	1.72
		368nm	0.334

For all three compounds, $\epsilon_{370} \cong 18{,}000$ and $\epsilon_{263} \cong 87{,}000$.

These spectra indicate that there is very little difference either in UV-visible absorbance or in molar extinction coefficients among these three compounds. In fact, within the limitations of experimental error, few or no spectral differences were observed.

The 1.6×10^{-5} M stock solutions of the three compounds were serially diluted 10-fold in 0.01 M sodium phosphate with 0.05% normal human serum at pH 4.8. They were also serially diluted 10-fold in PBS (pH 7.2) with 0.01% Tween 20®.

Because it is known that, in general, acridinium compounds are more stable at an acid pH, it was assumed that the counts obtained from the samples diluted in pH 4.8 buffer would be representative of the maximum stability with maximum chemiluminescnet output. All three compounds were serially diluted 10-fold to a final concentration of 1.6×10^{-10} M. A 10 μl aliquot of each sample was added to 90 μl of 0.05 N

HCl. Chemiluminescence was triggered with 200 µl of 0.03% H_2O_2 in 0.25 N NaOH and counts were monitored on a luminometer for 6 seconds with results as presented in Table 3. Results are presented in Tabl 3 for each of thr e runs.

TABLE 4

Compound No.	Identifying No.	Counts/6 Seconds
62	13513-211	92,669
		91,241
		91,995
83	30253-020	138,791
		141,962
		145,133
	13514-020	59,438
		59,443
		59,449

Within experimental error, chemiluminescent output on the luminometer did not differ among the compounds, as indicated in Table 4.

TABLE 5

Chemiluminescent Output at pH 4.8

Compound No.	Identifying No.	Counts/Mole
62	13513-211	5.7×10^{19}
83	30253-020	8.7×10^{19}
	13514-020	3.7×10^{19}

When 10 µl of these same compounds were diluted to 1.6×10^{-10} M in 90 µl PBS buffer (pH 7.2) with 0.01% Tween 20® and not acidified prior to running chemiluminescence output determinations as above, the results were somewhat different, especially for the acridinium carboxylate compound 13514-020, as shown in Table 5. Results are presented in Table 5 for each of three runs.

TABLE 6

Chemiluminescent Output at pH 7.2

Compound No.	Identifying No.	Counts/6 Seconds
62	13513-211	88,633
		89,135
		90,394
83	30253-020	133,560
		137,929
		142,299
	13514-020	8,185
		7,274
		6,363

The compound identified by the number 13514-020 produced only 4.4 X 10^{18} counts/mole in pH 7.2 buffer, almost an order of magnitude fewer counts than it produced at pH 4.8. This may be due to pseudobase formation by a large proportion of the molecules at the more alkaline pH, the pseudobase being substantially less chemiluminescent than the corresponding positively charged acridinium compound.

The N-sulfonylacridinium carboxamide compounds showed only a very small drop in counts when incubated at pH 7.2. This suggests that they do not undergo pseudobase formation to any appreciable degree, at least at this pH.

The dilution series of all three of the acridinium compounds in pH 7.2 buffer were stored overnight at room temperature and then assayed. Both N-sulfonylacridium carboxamide compounds showed virtually no change in chemiluminescence. The phenyl acridiunium carboxylate showed a significant drop after 20 hours at room temperature.

The samples were then placed in an incubator at 45°C. Every day for the duration of the study they were removed from the incubator, cooled to room temperature, and 10 µl aliquots diluted in 90 µl of PBS buffer (pH 7.2) were assayed for chemiluminescence.

Neither of the N-sulfonylacridinium carboxamides showed any significant difference in chemiluminescent output when diluted either in 0.05 N HCl or in PBS at pH 7.2. However, the acridinium carboxylate 13514-020 exhibited a significantly different chemiluminscent output when diluted in 0.05 N HCl or in PBS buffer at pH 7.2. When diluted in PBS buffer (pH 7.2), the acridinium carboxylate consistently produced at least 10-fold fewer counts than when diluted in 0.05 N HCl.

The 10,N-bis-(3-sulfopropyl) acridinium carboxamide (compound 83, 30253-020) appears to be quite stable at pH 7.2 at 45°C. After 10 days under such conditions no appreciable loss of chemiluminescence was observed. Compound 13513-211 produced 10-fold fewer counts, and the acridinium carboxylate 13514-020 produced 10^3 fewer counts under the same conditions.

Example 14

Preparation of Labeled IgG

Disulfopropyl compound 83, 30253-020, was activated by treatment with phosphorous oxychloride in acetonitrile at 45°C for 12 hours under argon. The solvent and excess $POCl_3$ were removed in vacuo and the activated compound was used directly in the labeling reaction.

Thus, 10 mg of rabbit IgG (Sigma Chemical Company, St. Louis, Missouri) was dissolved in 0.1 M sodium phosphate buffer (2 ml, pH 7.0) containing 1% Tween 80 ®. One ml of this solution was mixed with about 2 mg of the bis-sulfonylchloride. The solution was agitated periodically by sonication and stirring for one hour at room temperature.

An aliquot (0.5 ml) of th reaction solution was chromotographed over Sephadex G-25 (10 cm X 0.75 cm), as available from Pharmacia, Piscataway, New Jersey, and eluted with 0.1 M phosphate buffer (pH 6.5).

The labeled protein eluted as a weakly green fluorescent band. The labeled protein was further purified by HPLC using a Bio-Sil® TSK-250 column (BioRad, Richmond, California). The resulting conjugate (30253-34) contained 0.8 labels/protein, as determined from the ratio of the absorbance of 370 nm ($\epsilon \cong 10{,}000$, acridinium salt) to the absorbance 280 nm ($\epsilon \cong 210{,}000$, IgG).

Example 15

Heat Stability Studies

The conjugate 30253-34, as synthesized in Example 14, was serially diluted 10-fold n three buffers (0.1 M sodium phosphate, 0.01% Tween 20®, pH 6.3; 0.01 M sodium phosphate, 0.15 M NaCl, 0.01% Tween 20®, pH 6.8; and 0.01 M sodium phosphate, 0.15 M NaCl, 0.01% Tween 20®, pH 7.2) to a concentration of 2×10^{9} M IgG and 1.6×10^{9} M acridinium. A dilution series was prepared and initial counts were recorded by taking 10 µl of the sample, diluting with 90 µl of PBS buffer at pH 6.3, pH 6.8, or pH 7.2, and then triggering chemiluminescence with 200 µl of 0.03% H_2O_2 in 0.25 N NaOH. A 100 Ml sample of PBS buffer was used as a control for each series.

Counts shown in Table 6 are averages of results for duplicate samples assayed on the day on which the dilution series was prepared. The concentration shown in Table 5 is the concentration of the sample prior to dilution. The amount of parentheses for each entry in Table 5 is the amount of conjugate present in the sample.

TABLE 7

Concentration (Amount)	Counts/6 Seconds
pH 6.3	
buffer (0 moles)	253
2×10^{-10} M (2×10^{-14} moles)	216,054
1×10^{-10} M (1×10^{-14} moles)	100,842
5×10^{-11} M (5×10^{-15} moles)	48,704
2.5×10^{-11} M (2.5×10^{-15} moles)	23,771
1.25×10^{-11} M (1.25×10^{-15} moles)	11,475
6×10^{-12} M (6×10^{-16} moles)	5,866
pH 6.8	
buffer (0 moles)	233
2×10^{-10} M (2×10^{-14} moles)	295,608
1×10^{-10} M (1×10^{-14} moles)	149,725
5×10^{-11} M (5×10^{-15} moles)	76,820
2.5×10^{-11} M (2.5×10^{-15} moles)	38,801
1.25×10^{-11} M (1.25×10^{-15} moles)	18,408
6×10^{-12} M (6×10^{-16} moles)	9,398
pH 7.2	
buffer (0 moles)	726
2×10^{-10} M (2×10^{-14} moles)	309,445
1×10^{-10} M (1×10^{-14} moles)	156,311
5×10^{-11} M (5×10^{-15} moles)	77,238
2.5×10^{-11} M (2.5×10^{-15} moles)	39,879
1.25×10^{-11} M (1.25×10^{-15} moles)	19,925
6×10^{-12} M (6×10^{-16} moles)	10,526

Each dilution series was placed in a warm air incubator at 45°C after an initial reading was taken. A duplicate reading was made on each sample daily and then the readings were averaged.

When the conjugate was stored at pH 6.8 and at 45°C, there was no loss in chemiluminescent activity of the label over a 15 day period of observation, at any dilution. Essentially the same results were observed when the conjugate was stored in PBS buffer at pH 7.2.

Example 16

Comparison of CLIA vs. RIA

A. Preparation of Acridinium-Labeled Anti-HsAg Conjugate.

Compound 75 (13514-081, Example 6) (12.5 µmol) was dissolved in 200 µl of DMF, was treated with NHS (dissolved in 50 µl of DMF) and dicyclohexylcarbodiimide (dissolved in 50 µl of DMF) ("DCC"); and stirred for 12 hours at room temperature. The solution of the activated ester was mixed with mouse monoclonal anti-HBsAg in 0.1 M sodium phosphate buffer (pH 6.3) in a molar ratio of 100:1 at 4°C for 12 hours.

The conjugate was then dialysed against PBS buffer, pH 6.3, until the absorbance of the dialysate indicated no free label. A UV spectral analysis indicated between 2 to 6 labels/antibody (as determined from a ratio of absorbances as in Example 14).

B. Assay for HBsAg.

Either type A_d or type A_y HBsAg (200 µl) was diluted in calf serum and was reacted with an AuszymeTM (Abbott Laboratories, Abbott Park, Illinois) monoclonal antibody bead and 2 X 10^5 of counts of ^{125}I-labeled mouse monoclonal anti-HBsAg antibody (40 µl, in the RIA) or an acridinium-labeled mouse monoclonal anti-HBsAg antibody (40 µl, in the CLIA) in PBS containing 50% calf serum, 10% human serum, 0.05% Tween 20® and 5 mM EDTA (pH 6.3), for three hours at 40°C. The beads were then washed 6 times in water and counted for their activities. Calf serum was used as a negative control.

In the CLIA, a polystyrene bead with conjugate bound adsorbed thereto was mixed with 250 µl phosphate, 0.5 mM, pH 5.3, in a glass vial suitable for use in a luminometer. While the sample was in the measuring position, 0.2 ml of 0.03% H_2O_2 in 0.25 N NaOH was then injected into the glass vial. The light emmitted was measured in the luminometer. Reading began 0.012 seconds before initiation of the chemical reaction and continued for 6 seconds.

The results are presented in Table 7.

TABLE 8

Concentration (ng/ml)	CLIA		RIA	
	A_d	A_y	A_d	A_y
1.0	2214	3144	371	400
0.5	1256	2494	236	408
0.25	701	921	221	248
0.125	521	592	173	179
Calf Serum	151	179		
Cut-off	327	376		

Under the stated conditions, the sensitivity for the CLIA was less than 0.125 ng/ml for both the A_d and A_y types of HBsAg. For the RIA the sensitivity was 1.0 ng/ml for both the A_d and A_y types. The cut-off count was 2.1 times that of the negative control.

Table 8 clearly shows that chemiluminescent immunoassays according to the present invention are more sensitive than comparable radioimmunoassays.

Example 17

A comparison of CLIA and EIA

A. Preparation of lab led anti-hTSH (30234-207).

Compound 75 (13514-081, Example 6) (2 mg, 4.3 µmoles) in 200 ml of acetonitrile was treated with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (Sigma, St. Louis, Missouri) (10 µmoles) in 100 µl of acetonitrile and N-hydroxysuccinimide (4-0 µmoles) in 100 µl of acetonitrile for 12 hours at 25°C in the dark.

The active ester was mixed with anti-hTSH in PBS buffer containing 0.5% 3-[(3-cholamidopropyl) dimethylammonio]-1-propane-sulfonate ("CHAPS") at pH 6.5 in a ratio of 50:1 (antibody:active ester). After coupling for 3 hours at 25°C, the labeled antibody was dialysed against PBS buffer containing 0.5% CHAPS at pH 6.5 until no free label was present in the dialysate by U.V.

Based on the U.V. spectra, the conjugate had an average of 10 labels per antibody.

B. Assay for hTSH.

CLIA and EIA were compared using the Abbott hTSH-EIA Kit (Abbott Laboratories, Abbott Park, Illinois) with the exception that for the CLIA, the anti-hTSH acridininium conjugate was used in place of the kit anti-hTSH-HRPO conjugate. Thus, a standard curve was generated by incubating the kit standards with the kit beads at 37°C for 1 hour, then washing three times. For the CLIA, the conjugate prepared above was diluted 1:5000 with PBS buffer containing 50% calf serum, 1% normal mouse serum, 0.05% Tween® 20 and 2 mM EDTA at pH 6.3. One hundred microliters of this solution was incubated with the beads for 1 hourw at 37°C, then washed four times.

The beads were transferred one by one to the reaction vial of a luminometer containing 400 µl of water and reacted with 200 µl of 0.03% H_2O_2 in 0.2 N NaOH. Photon counts were recorded for 6 seconds.

The EIA was carried out according to the instructions in the kit insert on a Quantum II® spectro photometer (Abbott Laboratories, Abbott Park, Illinois)

The results are shown in Table 9.

TABLE 9

Concentration (µIu/ml)	CLIA (counts)	EIA (A_{492})
0	533(SD35.4)	0.012
1	5064	0.062
4	14476	0.176
10	32092	0.397
25	66072	0.828
60	110,984	1.602

Under these conditions the sensitivity of the CLIA was 0.016 µIU/ml (0 standard + 2 SD) while the EIA had a sensitivity of 0.05 µIU/ml.

Example 18

Preparation of 5-methyl-6-[N-tosyl-N-(2-carboxyethyl)]-phenanthridiniumcarboxamide

Phenanthridine-6-carboxylic acid (400 mg, 1.8 mmoles) [pr pared by the metod of Wittig et al., Justus Li big's Ann., 577, 1 (1952)], was suspended in methylene chloride (20 ml, distilled from P_2O_5) and cooled to 0°C under nitrogen. Oxalyl chloride (320 µl, 3.6 mmoles) (Aldrich Chemical Co., Milwaukee, Wisconsin) was added, followed by DMF (5 µl). As the reaction mixture was stirred for on hour at 0°C and for 30

minutes at 25°C, all the carboxylic acid dissolved. The solution was evaporated to dryness to give the acid chloride which was used without further purification.

Methyl N-tosyl-β-alanine was prepared from methyl-β-alanine (Aldrich Chemical Company, Milwaukee, Wisconsin) and tosyl chloride (Aldrich Chemical Company, Milwaukee, Wisconsin) according to the procedure of Example 1. Potassium t-butoxide (600 mg, 5.4 mmoles, freshly sublimed) was added to a solution of 1.3g (5.4 mmoles) of methyl N-tosyl-β-alanine in 50 ml of THF. After stirring for 15 minutes and at room temperature and under N_2, the suspension was evaporated to dryness. The potassium salt of methyl N-tosyl-β-alanine, was resuspended in 20 ml of THF, mixed with the acid chloride (in 20 ml of THF), and stirred for 12 hours.

The resulting suspension was poured into 100 ml of ethylacetate, washed with 50 ml of water and washed twice with 25 ml of brine. After drying over $MgSO_4$ and evaporating to dryness, the residue was chromatographed on a ChromatatronTM chromatograph (available from Harrison Research, Palo Alto, California) using a 4 mm silica rotor and employing a 25/75 ethylacetate/hexane gradient. The product (R_f0.2) was collected, then recrystallized from benzene/hexane (i.e., the product was dissolved in benzene, and hexane was added until cloudy) to give 130 mg of methyl 6-[N-tosyl-N-(2-carboxyethyl)]-phenanthridinecarboxamide, Compound 84, 13514-225.

Compound 84, 13514-225, was methylated according to the procedure in Example 3 to give methyl 5-methyl-6-[N-tosyl-N-(2-carboxyethyl)]-phenanthridiniumcarboxamide, compound 85, 13514-227. Compound 85 was hydrolyzed according to the procedure in Example 8 to provide 5-methyl-6-[N-tosyl-N-(2-carboxyethyl)]-phenanthridiniumcarboxamide, compound 86, 13514-228.

Compouns 84, 85 and 86 are described using the numerals, symbols and abbreviations as explained in Example 1.

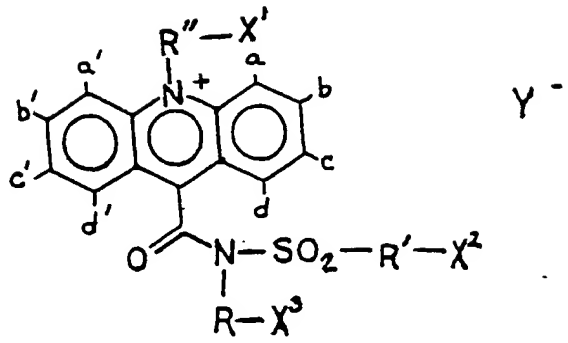
84. 13514-225 Methyl 6-[N-tosyl-N-(2-carboxyethyl)]-phenanthridinecarboxylate
MS M + H @ 463

85. 13514-227 Methyl 5-methyl-6-[N-tosyl-N-(2-carboxyethyl)]-phenanthridiniumcarboxamide
MS M^+ @ 477
Mp 136°C

86. 13514-228 5-Methyl-6-[N-tosyl-N-(2-carboxyethyl)]-phenanthridiniumcarboxamide
MS M^+ @ 463

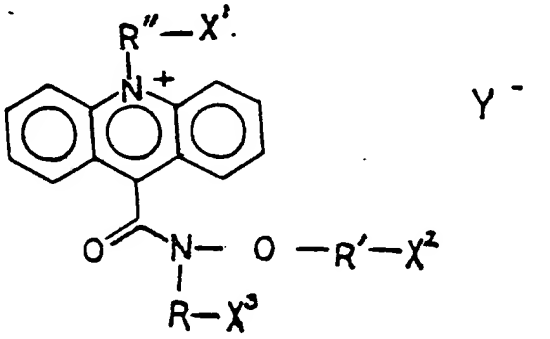
Although the present invention has been described in terms of preferred embodiments, it is understood that modifications and improvements will occur to those skilled in the art.

For example, in light of the results presented herein, it is expected that additional compounds which are useful according to the present invention may be identified by the formula

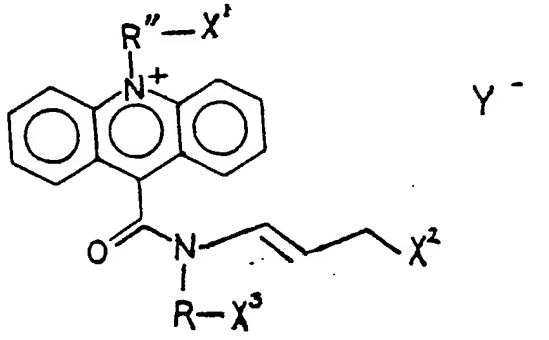


wherein a, b, c, d, a', b', c', d' independently may be hydrogen, alkyl, aryl, amino, substituted amino, carboxy-alkyl, sulfoalkyl, alkoxyl, aryloxy, sulfo, thio alkoxyl, thioaryloxy aminoalkyl, protected aminoalkyl, hydroxyalkyl, protected hydroxyalkyl, haloalkyl, or any adjacent of these positions may be linked so as to form aromatic rings fused to the acridine nucleus.

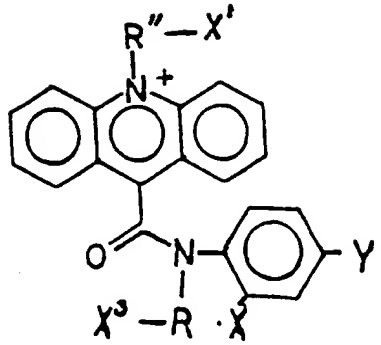
In addition, Sheehan et al., U.S. Patent No. 3,539,574 described chemiluminescent acridinium compounds which are also expected to be useful according to the present invention. Other isomeric acridinecarboxylic acids, quinoline carboxylic acids, isoquinoline carboxylic acid, other activated acrdine amides, and other activated acridine esters are expected to be useful according to the present invention. Such compounds include, without limitation: hydroxamates identified by the formula



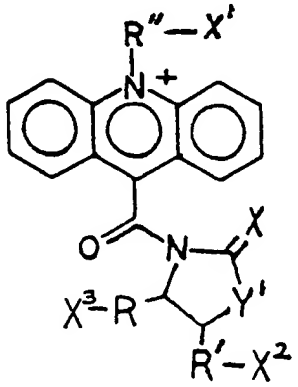
enamides identified by the formula



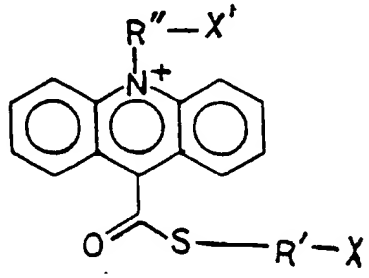
arylamides identified by the formula



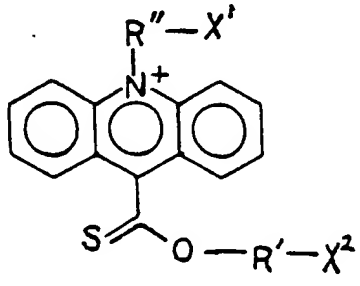
wherein X and Y¹ are electron withdrawing groups; N-heterocycles identified by the formula



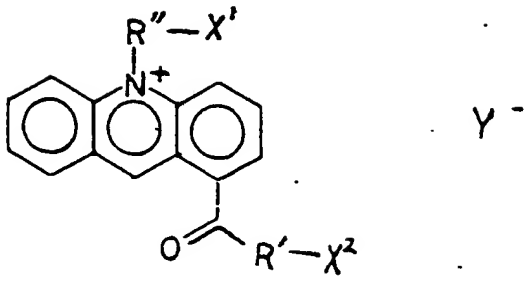
wherein X and U¹ may independently be O, S, P, N, or C; activated esters such as thiolesters identified by the formula



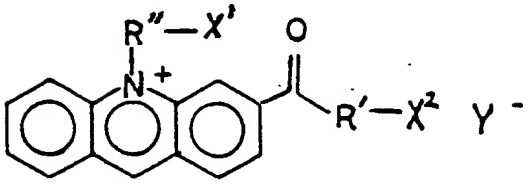
or such as thioesters identified by the formula



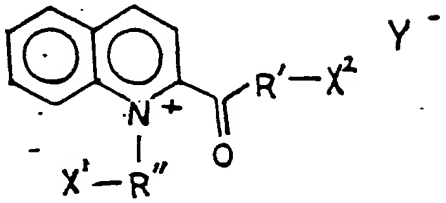
acridine acids id ntifi d by the formula



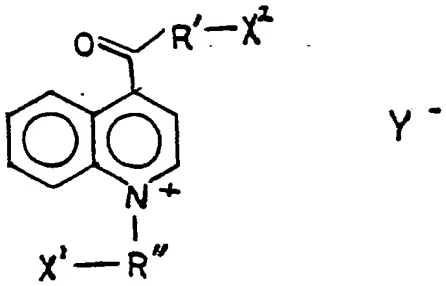
or by the formula



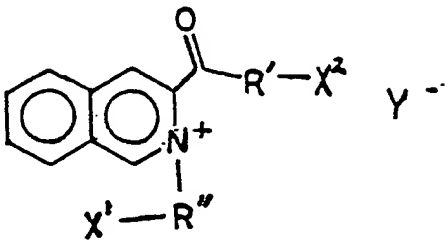
quinoline acids identified by the formula



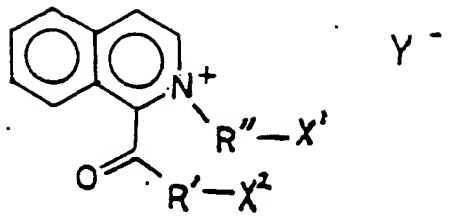
or by the formula



or isoquinoline acids identified by the formula



or by the formula



It is understood that those skilled in the art will be enabled by the above specification to incorporate reactive functional groups for attaching the label to an analyte into compounds according to the present invention.

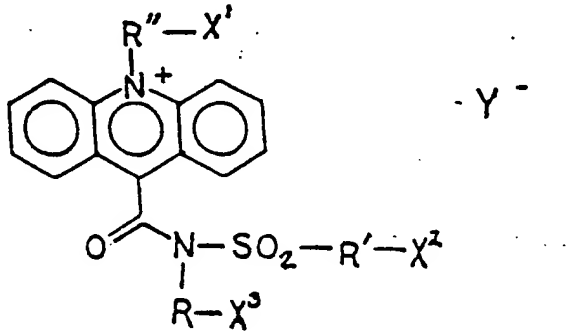
It is also contemplated that compounds according to the present invention will be: used in labeling DNA probes; incorporated into an enzyme substrate wherein the product of the enzymatic reaction is the chemiluminescent compound; and incorporated into systems which involve energy transfer or fluorescent quenching.

Compounds according to the present invention may also be: incorporated into a system which employs the compound as a labeling reagent in a post-column HPLC detection system; used to measure H_2O_2 concentration; and used as a source of intense pulsed light.

Therefore, it is intended that the present invention include all such variations and improvements as come within the scope of the invention as claimed.

Claims

1.a chemiluminescent compound identified by the formula



and isomers thereof wherein R, R', R", X^1, X^2, and X^3 are substituents which do not interfere with effective chemiluminescence with the proviso that R-X^3, R'-X^2 and R"-X^1 may independently be hydrogen.

2. The chemiluminescent compound as recited in claim 1:
wherein R, R', and R" are spacer arms;
wherein X^1, X^2 and X^3 are independently members of the group consisting of hydrogen, carboxy, carboalkoxyl, carboxamido, carboaryloxy, cyano, carboximido, isocyanato, isothiocyanato, sulfo, sulfonyl halide, carbonyl halide, N-succinimidylcarboxy and N-maleimide groups; and
wherein Y is an appropriate counter ion.

3. The chemiluminescent compound as recited in claim 2 wherein Y is a counter ion selected from the group consisting of sulfate, alkylsulfate, halosulfate, haloborate, haloacetate, halophosphate, phosphate and halide.

4. The chemiluminescent compound as recited in claim 1 wherein R, R', and R" independently comprise a member selected from the group consisting of alkyl, alkylene, aryl, substituted alkyl, substituted alkylene and substituted aryl groups such that:
one or more hydrogens of said member is replaced by an alkyl, aryl, alkylene, substituted alkyl, substituted alkylene, substituted, aryl, alkoxy, aryloxy, halo, amino protected amino, substituted amino hydroxy, protected hydroxy, oxo, thio, imino, mercapt or substituted mercapto group;
or such that one or more carbon atoms of the member is replaced by a h teroatom.

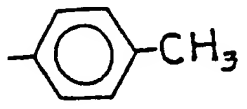
5. The chemiluminescent compound as recited in claim 4 wherein said heteroatom is selected from the group consisting of nitrogen, phosphorus, sulfur and oxygen.

6. The chemiluminescent compound as recited in claim 2 wh rein R, R', and R" independently are spacer arms of the formula

$-(CH_2)_n-$

where n = 0 - 50.

7. The chemiluminescent compound as recited in claim 1 wherein R" is $-CH_2-$, X^1 is -H, and $R'-X^2$ is identified by the formula

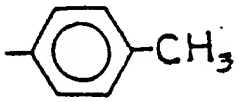


8. The chemiluminescent compound as recited in claim 7 wherein said compound is identified by the formula 10-methyl-N-[2-carboxyethyl]-N-tosyl-9-acridinium carboxamide.

9. The chemiluminescent compound as recited in claim 7 wherein said compound is identified by the formula 10-methyl-N-(4-carboxybutyl)-N-tosyl-9-acridinium carboxamide.

10. The chemiluminescent compound as recited in claim 7 wherein said compound is identified by the formula 10-methyl-N-(5-carboxypentyl)-N-tosyl-9-acridinium carboxamide.

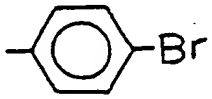
11. The chemiluminescent compound as recited in claim 1 wherein formula R" is $-(CH_2)_3-$, X^1 is $-SO_3-$, and $R'-X^2$ is identified by the formula



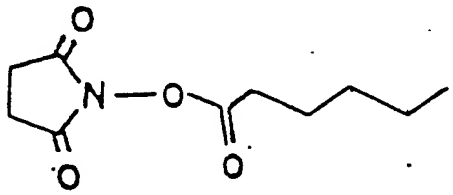
12. The chemiluminescent compound as recited in claim 11 wherein said compound is identified by the formula 10-(3-sulfopropyl)-N-(2-carboxyethyl)-N-tosyl-9-acridinium carboxamide.

13. The chemiluminescent compound as recited in claim 11 wherein said compound is identified by the formula 10-(3-sulfopropyl)-N-(3-sulfopropyl)-N-tosyl-9-acridinium carboxamide.

14. The chemiluminescent compound as recited in claim 7 wherein $R'-X^2$ is identified by the formula,



and wherein $R-X^3$ is identified by the formula



15. The chemiluminescent compound as recited in claim 7 wherein said compound is selected from 10-methyl-N-phenyl-N-tosyl-9-acridinium carboxamide trifluoromethanesulfonate, 10-methyl-N-phenyl-N-(p-bromobenzenesulfonyl)-9-acridinium carboxamide trifluoromethanesulfonate, 10-methyl-N-phenyl-N-(p-nitrobenzenesulfonyl)-9-acridinium carboxamide trifluoromethanesulfonate, 10-methyl-N-phenyl-N-(o-nitrobenzenesulfonyl)-9-acridinium carboxamide trifluoromethanesulfonate, or 10-methyl-N-phenyl-N-trifluoromethanesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate.

16. The chemiluminescent compound as recited in claim 7 wherein said compound is identified by the formula 10-methyl-N-isopropyl-N-tosyl-9-acridinium carboxamide trifluorom thanesulfonate,10-methyl-N-isopropyl-N-(p-bromobenzenesulfonyl)-9-acridinium carboxamide trifluoromethanesulfonate, 10-methyl-N-isopropyl-N-(o-nitrobenzenesulfonyl)-9-acridinium carboxamide trifluorom than sulfonate, or 10-methyl-N-isopropyl-N-tifluoromethanesulfonyl-9-acridinium carboxamid trifluoromethanesulfonate.

17. The chemiluminescent compound as recited in claim 7 wherein said compound is identified by the formula 10-methyl-N-butyl-N-(2,4,6 trimethylbenzenesulfonyl)-9-acridinium carboxamide trifluoromethanesulfonate, 10-methyl-N-butyl-N-(2,4,6-tri-isopropyl-benzenesulfonyl)-9-acridinium carboxamide trifluoromethanesulfonate, 10-methyl-N-phenyl-N-tosyl-9-acridinium carboxamide trifluoromethanesulfonate, 10-methyl-N-butyl-N-(p-bromobenzenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate, 10-methyl-N-butyl-N-(o-nitrophenylsulfonyl)-9-acridinium carboxamide trifluoromethanesulfonate, 10-methyl-N-butyl-N-(p-nitrobenzenesulfonyl)-9-acridinium carboxamide trifluoromethanesulfonate, 10-methyl-N-butyl-N-(2,4-dinitrobenzenesulfonyl)-9-acridinium carboxamide trifluoromethanesulfonate, or 10-methyl-N-allyl-N-tosyl-9-acridinium carboxamide trifluoromethanesulfonate.

18. The chemiluminescent compound as recited in claim 1 wherein said compound is identified by the formula methyl 6-[N-tosyl-N-(2-carboxyethyl)]-phenanthridinecarboxamide, methyl 5-methyl-6-[N-tosyl-N-(2-carboxyethyl)]-phenanthridiniumcarboxamide, or 5-methyl-6-[N-tosyl-N-(2-carboxyethyl)]-phenanthridiniumcarboxamide.

19. A method for preparation of a chemiluminescent compound comprising the steps of:

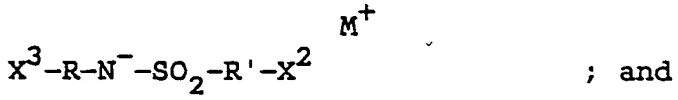
contacting an amine identified by the formula

$R\text{-}X^3NH_2$

with a sulfonylhalide identified by the formula

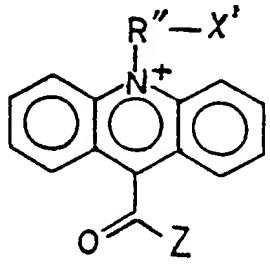
$W\text{-}So_2\text{-}R'\text{-}X^2$

in an inert solvent in the presence of base to form a sulfonamide anion and metal ion identified by the formulas



; and

a) acylating with an activated 0-acridinecarboxylic acid identified by the formula



wherein R, R′ and R″ are independently selected from the group consisting of: alkyl, aryl, alkylene, substituted alkyl, substituted alkylene and substituted aryl, alkoxy, aryloxy, halo, amino, protected, amino, substituted amino hydroxy, protected hydroxy, oxo, thio, imino, mercapto groups; and alkyl, aryl, alkylene substituted alkyl, substituted alkylene and substituted aryl groups comprising a heteroatom selected from the group consisting of nitrogen, phosphorus, sulfur and oxygen with the proviso that R-X^3, R′X^2, and R″-X^1 may independently be hydrogen;

wherein X^1, X^2, and X^3 are independently members of the group consisting of hydrogen, carboxy, carboalkoxyl, carboxamido, carboaryloxy, cyano, carboximido, isocyanato, isothiocyanato, sulfo, sulfonyl halide, carbonyl halide, N-carboxysuccinimide and N-maleimide groups;

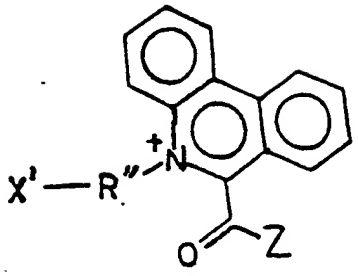
wherein Y is an appropriate counter ion;

wherein W is selected from the group consisting of chloro and fluoro groups; and

wherein M is selected from the group consisting of Li, Na and K; and

wher in Z is selected from the group consisting of halo, imidazolo, N-hydroxysuccinimidyl and azido groups; or

b) acylating with an activated phenanthridine-6-carboxylic acid identified by the formula



wherein R, R′ and R″ are independently selected from the group consisting of: alkyl, aryl, alkylene, substituted alkyl, substituted alkylene and substituted aryl, alkoxy, aryloxy, halo, amino, protected amino, substituted amino hydroxy, protected hydroxy, oxo, thio, imino, mercapto groups; and alkyl, aryl, alkylene substituted alkyl, substituted alkylene and substituted aryl groups comprising a heteroatom selected from the group consisting of nitrogen, phosphorus, sulfur and oxygen with the proviso that R-X^3, R′-X and R″X^1 may independently be hydrogen;

wherein X^1, X^2 and X^3 are independently members of the group consisting of hydrogen, carboxy, carboalkoxyl, carboxamido, carboaryloxy, cyano, carboximido, isocyanato, isothiocyanato, sulfo, sulfonyl halide, carbonyl halide, N-carboxysuccinimide and N-maleimide groups;

wherein Y is an appropriate counter ion;

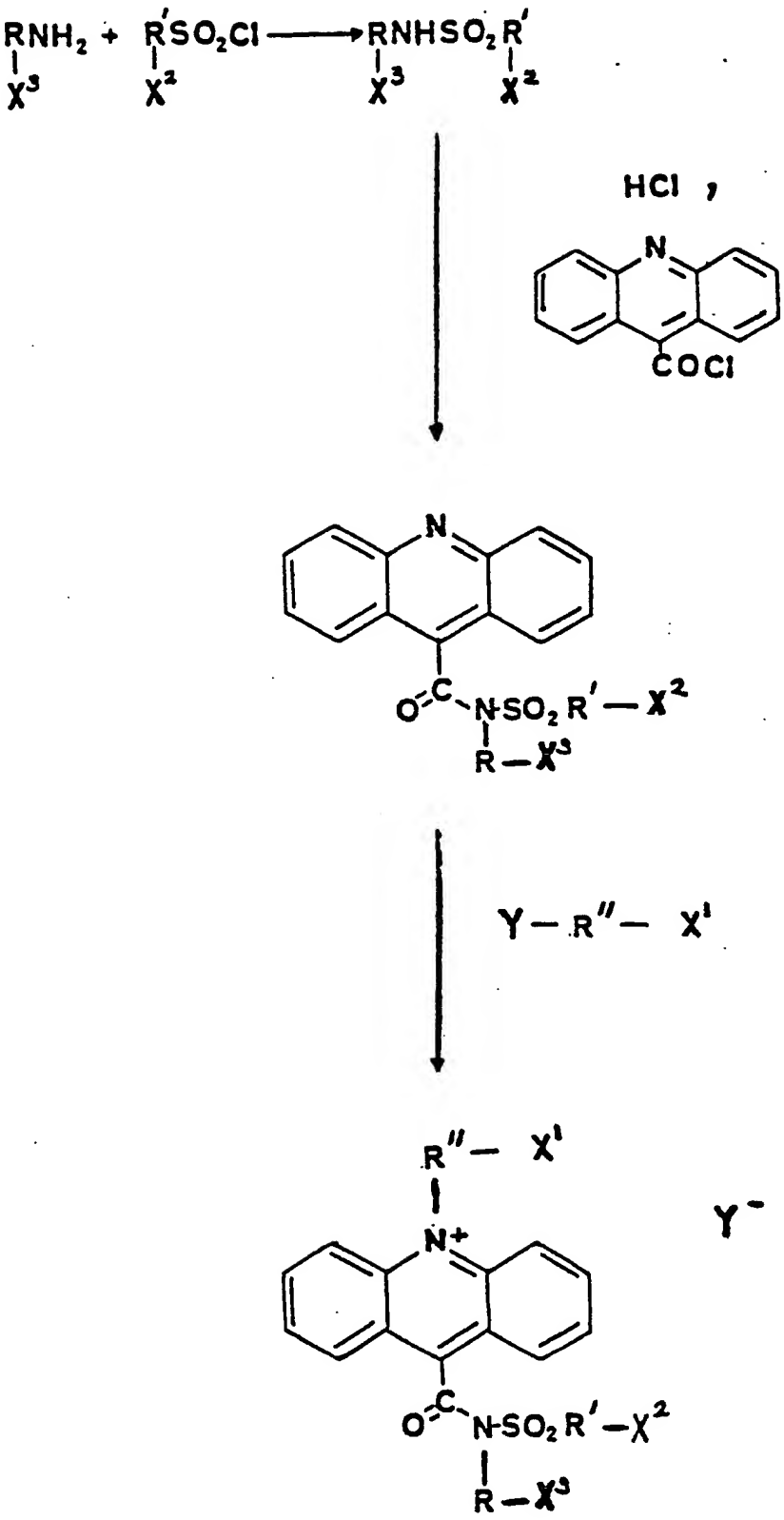
wherein W is selected from the group consisting of chloro and fluoro groups; and

wherein M is selected from the group consisting of Li, Na and K, and

wherein Z is selected from the group consisting of halo, imidazolo, N-hydroxysuccinimidyl and azido groups.

20. A conjugate formed by an antibody or antigen conjugated to a chemiluminescent compound as recited in claim 1.

21. A method for performing a chemiluminescent immunoassay to test for the presence of an antigen or antibody to an antigen as recited in claim 20 comprising the step of exposing a sample to a conjugate as recited in claim 20.


RNH2 + R'SO2Cl → RNHSO2R'
X3 X2 X3 X2
HCl ,
N
COCl
N
O=C
N-SO2 R' — X2
R — X3
Y — R'' — X1
R'' — X1
N+
Y-
O=C
N-SO2 R' — X2
R — X3

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Description

Background

The present invention relates in general to chemiluminescent methods and materials and in particular to methods and materials involving chemiluminescent acridinium and phenanthridinium salts.

Chemiluminescence may be defined as the generation of light from a chemical reaction. The mechanism of most chemiluminescent reactions is not known in detail, but a generalized mechanism [Schuster et al., Advances in Physical Organic Chemistry, 187-238 (1984)] may be outlined:

$$A \rightarrow B^* \rightarrow B + h\nu$$

Compound A undergoes a chemical reaction (usually oxidation) to yield a product in an electronically excited State ("B*"). As it returns to the ground state ("B"), this product gives up energy in the form of light ("$h\nu$").

Although competing dark reactions may decrease the efficiency of the overall reaction to less than 1%, some bioluminescent systems may achieve 60-70% efficiency, and, in many cases, limits of detection in the femtomole (10^{-15} mole) to attomole (10^{-18} mole) range have been recorded.

Chemiluminescence has been used for a variety of purposes in analytical chemistry where other methods fail to have adequate sensitivity. In immunodiagnostics, chemiluminescent immunoassays ("CLIA") may thus match or exceed the sensitivity of radioimmunoassays ("RIA") or enzyme immunoassays ("EIA") [Kircka et al., Diagnostic Medicine, 1, 45-52 (1984)].

Luminol and isoluminol derivatives are the most widely used chemiluminescent reagents for immunoassays. The light-yielding reaction is initiated by oxidation with alkaline hydrogen peroxide in the presence of catalysts such as microperoxidase or transition metal ions. Light emission occurs at about 465 nm, which corresponds to the fluorescence emission of the product, aminophthalic acid. Aminobutylethyl isoluminol ("ABEI") may be used as a label in immunoassays and is commercially available.

A second group of chemiluminescent reagents, aryl oxalates [Gill, Aldrichimica Acta, 16, 59-61 (1983) and Catherall et al., J. Chem. Soc. Faraday Trans. 2, 80, 823-834 (1984)], have been used as commerical cold light sources [see e.g., Tseng et al., U.S. Patent No. 4,338,213] and in high performance liquid chromatography ("HPLC") detectors [Kobayashi et al., Anal. Chem., 52, 424-427 (1980) and Miyaguchi et al., J. Chromatogr., 303, 173-176 (1984)]. It is thought that these derivatives react with hydrogen peroxide in buffered or unbuffered solvents to give a dioxetan-dione which decomposes quickly to give CO_2 in an excited state. Energy is then transferred by electron transfer to a fluorescer molecule which emits light.

A third group of reagents, 10-methyl-acridinium-9-carboxylic acid aryl esters, are chemiluminescent in the presence of alkaline hydrogen peroxide and in the absence of a catalyst. The mechanism is thought to involve initial attack by a hydroperoxide anion, followed by intramolecular displacement of the phenolate (the "leaving group") to give a strained dioxetan-one. The strained dioxetan-one decomposes to CO_2 and excited N-methyl-acridone, which emits light at 430 nm. Carboxy-substituted acridinium salts have been used as labels in immunoassays [Weeks et al., Clin. Chem., 29, 1474-79 (1983); Campell et al., European Patent Application No. 82,636; and McCapra et al., UK Patent No. GB 1,461,877]. Also, 5-methyl-phenanthridinium-6-carboxylic acid aryl esters, which are isomeric with the acridinium aryl esters, have been used as labels in immunoassays [Lin et al, European Patent Application No. 170,415].

Despite their usefulness in immunoassays, antibody-conjugated phenyl 10-methyl-9-acridiniumcarboxalates, in our hands, are unstable due to hydrolysis above pH 4.0 (-20 °C to 40 °C), losing greater than 10% of their activity within three days. Although acridinium esters are stable below pH 4.0, conjugate antibodies are often not stable in this pH range.

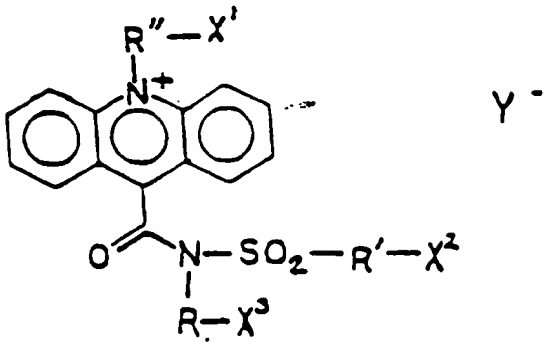
In Tseng et al., supra, bis-N-alkyl-N-trifluoromethyl sulfonyl oxalamides are indicated to be more stable than the corresponding aryl esters and are also indicated to be as efficient. The nucleofugacity of the phenol and the trifluoromethyl sulfonamide are indicated to be comparable, i.e. it is indicated that each has a pK_a of about 7. Gill, supra, "look forward" to the development of a particular sulfonyl oxalamide as an example of an oxalate with "higher" quantum efficiency.

Brief Description of the Drawings

The Figure illustrates the synthesis of a 10-alkyl-N-sulfonyl-9-acridinium carboxamide according to the present invention.

Summary of the Invention

The present invention provides chemiluminescent compounds identified by the formula



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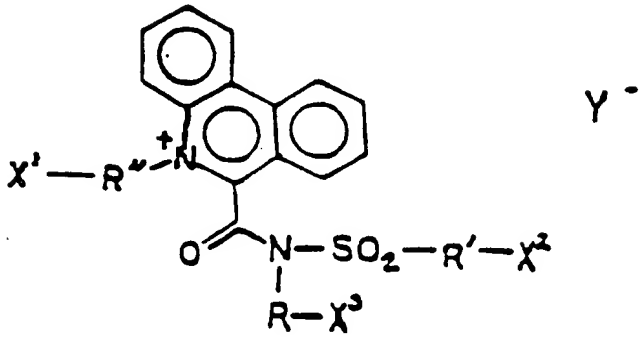


wherein R, R', and R'' may independently include a member selected from the group consisting of alkylene, arylene, substituted alkylene, and substituted arylene groups, such that: one or more hydrogens of said member is replaced by an alkyl, aryl, substituted alkyl, substituted aryl, alkoxy, aryloxy, halo, amino, protected amino, substituted amino, hydroxy, protected hydroxy, oxo, thio, imino, mercapto or substituted mercapto group; or such that one or more carbon atoms of the member is replaced by a heteroatom;

wherein X^1, X^2, and X^3 are independently members of the group consisting of hydrogen, carboxy, carboalkoxyl, carboxamido, carboaryloxy, cyano, carboximido, isocyanato, isothiocyanato, sulfo, sulfonyl halide, carbonyl halide, N-succinimidyloxycarboxy and N-maleimide groups; or

wherein one of R'-X^2 or R-X^3 can either be a nitro-benzene, provided that the other one is selected from phenyl, iso-propyl, n-butyl or benzyl 5-carboxypentyl, or a dinitro-benzene, provided that the other one is selected from n-butyl and phenyl; and

wherein Y^- is an appropriate counterion; with the proviso that R-X^3, R'-X^2 and R''-X^1 may be independently hydrogen, and

with the further proviso that when in the compounds of formula I in either one of R'-X^2 and R-X^3, X^2 or X^3 is selected from carbopentachlorophenoxy, carbo-p-nitrophenoxy, carboximido, isothiocyanate, N-maleimide and N-succinimidylcarboxy, and the other one of R'-X^2 and R-X^3 is selected from hydrogen, alkyl, aryl or benzyl, or such aryl or benzyl substituted by alkoxy, aryloxy, amino, or hydroxy,

then X^1 is different from H and R''-X^1 is different from H;

and also selected from 10-methyl-N-allyl-N-p-toluenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate.

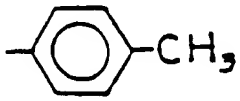
The counterion Y^- may be selected from the group consisting of sulfate, alkylsulfate, halosulfate, haloborate, haloacetate, halophosphate, phosphate and halide.

The heteroatom may be selected from the group consisting of nitrogen, phosphorus, sulfur and oxygen. R, R', and R'' independently may also be spacer arms of the formula

$-(CH_2)_n-$

where n = 0 - 50. Specifically, R'' may be $-CH_2-$ and X^1 may be -H.

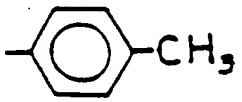
Illustrative of compounds according to the present invention are those wherein R'' is $-CH_2-$, X^1 is -H, and R'-X^2 is identified by the formula



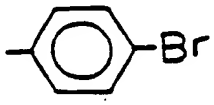
An example of such compounds is 10-methyl-N-(4-carboxybutyl)-N-tosyl-9-acridinium carboxamide.

Another example of such compounds is 10-methyl-N-(5-carboxypentyl)-N-tosyl-9-acridinium carboxamide.

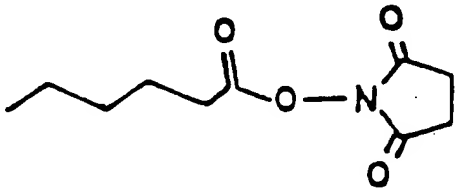
Further illustrative of compounds according to the present invention are those wherein R'' is $-(CH_2)_3-$, X^1 is $-SO_3-$, and R'-X^2 is identified by the formula



Still further illustrative of compounds according to the present invention are those wherein R'-X^2 is identified by the formula,



and wherein R-X^3 is identified by the formula



The currently most preferred compounds according to the present invention for use in chemiluminescent immunoassays are 10-methyl-N-[2-carboxyethyl]-N-tosyl-9-acridinium carboxamide, 10-(3-sulfopropyl)-N-(2-carboxyethyl)-N-tosyl-9-acridinium carboxamide and 10-(3-sulfopropyl)-N-(3-sulfopropyl)-N-tosyl-9-acridinium carboxamide.

A method, according to the present invention, for preparation of a chemiluminescent compound includes the steps of contacting an amine identified by the formula

X^3-R-NH_2

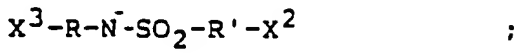
with a sulfonylhalide identified by the formula

$W\text{-}SO_2\text{-}R'\text{-}X^2$

in an inert solvent in the presence of base to form a sulfonamide identified by the formula

$X^3RNHSO_2R'X^2$; and

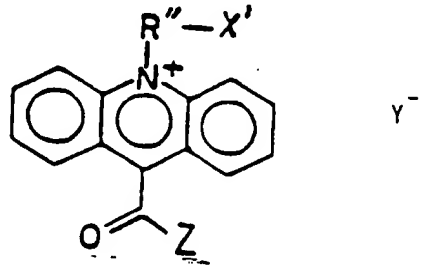
contacting the sulfonamide in an inert solvent in the presence of a base to form a sulfonamide anion identified by the formula



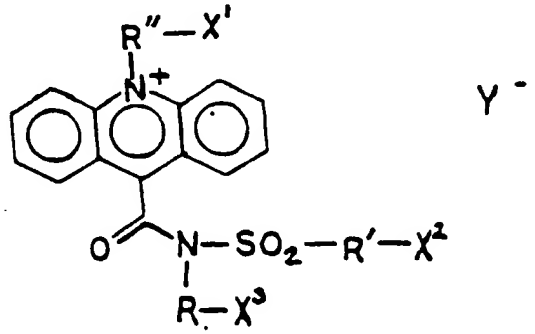
;

and

a) acylating with an activated 9-acridinecarboxylate compound identified by the formula

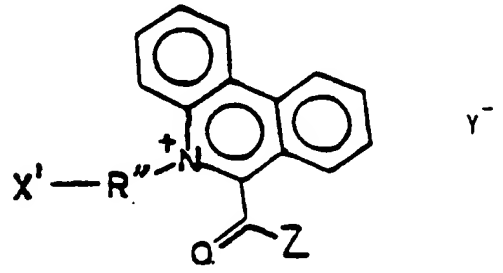


to produce said chemiluminescent compound identified by the formula

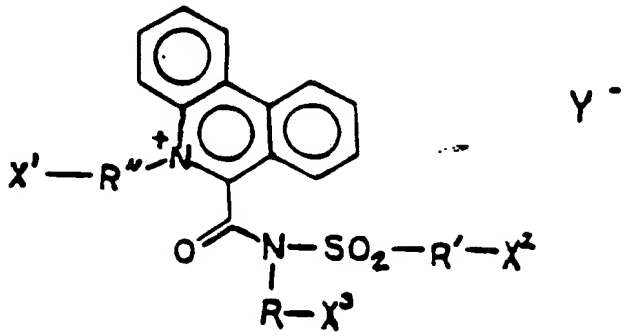


or

b) acylating with an activated phenanthridine-6-carboxylate compound identified by the formula

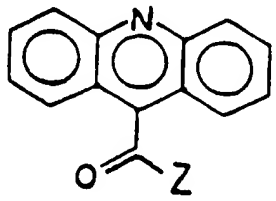


to produce said chemiluminescent compound identified by the formula

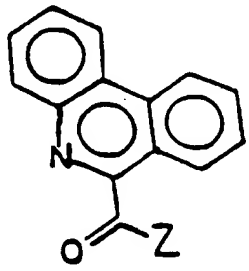


wherein W is selected from the group consisting of chloro and fluoro groups, wherein M is selected from the group consisting of Li, Na and K, wherein the activating group Z is selected from the group consisting of halo, imidazolo, N-hydroxysuccinimidyl and azido groups and wherein all other symbols are as defined above.

Furthermore, a method according to the present invention can comprise steps wherein said acylation is carried out with



in part a) and



in part b) and R''-X^1 is subsequently attached through alkylation with Y-R''-X^1 wherein Y on reaction becomes said counter ion.

A conjugate according to the present invention may be formed by covalently coupling an antibody, a hapten, an antigen or a polynucleotide (e.g., DNA or RNA) to a chemiluminescent compound according to the present invention, and a method for performing a chemiluminescent assay comprises the step of exposing a sample to be tested to the conjugate in order to detect the pr sence of a substance specifically reactive with the conjugate, e.g., a specific antigen, a specific antibody or a complementary polynucleotide (i.e., a polynucleotide which forms sequence-specific hydrogen bonds with the polynucleotide conjugate according to the present invention).

Representative of conjugates according to the present invention are antibodies or antigens coupled to a chemiluminescent compound of the present invention.

Repr sentative of immunoassays according to the invention are those testing for the presence of antigens comprising the step of exposing a sample to the corresponding antibody-chemiluminescent compound conjugate and thos testing for the presence of antibodies comprising the step of exposing a sample to the corresponding antigen-chemiluminescent compound conjugate.

Detailed Description

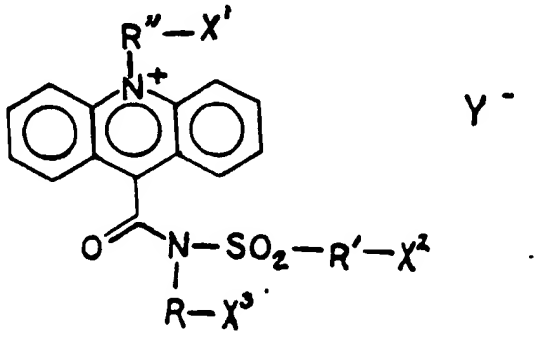
The problem of acridinium aryl ester instability is approached in the present invention by changing the leaving group from a phenolate to a sulfonamide anion. While both leaving groups have a pK_a of about 10, the acridinium sulfonylamide has the additional stabilization associated with amide bonds. This is reflected in a comparison in the infrared of the carbonyl stretching frequency of the aryl ester (1730 cm^{-1}) with that of the sulfonylamide (1680 cm^{-1}).

A class of acridinium salts, 10-alkyl N-alkyl (aryl) sulfonyl-N-alkyl(aryl) 9-acridinium carboxamide salts, was prepared according to the general scheme illustrated in the Figure. In the Figure, R, R' and R" are substitutents which may function as spacer arms, solubility modifiers and/or reactivity modifiers but which do not interfere with the chemiluminescent reaction. ("Interfere" is defined herein to mean "prevent the production of effective chemiluminescence", i.e., prevent production of chemiluminescence to the extent that the compound is not useful for the intended application.) Also in the Figure, X^1, X^2, X^3 are substituents which may function as solubility enhancers and/or as reactive groups for linkage to an analyte or as groups which may be readily converted to such reactive or linker groups by means well known to those skilled in the art. Y^- is a counterion in the Figure.

Salts produced according to the scheme of the Figure have generated light upon oxidation with alkaline hydrogen peroxide. The compounds were made from readily available amines (X^3-RNH_2) and sulfonyl chlorides (X^2-$R'SO_2Cl$). When acylated with 9-chlorocarbonyl acridine, the intermediate sulfonamide (X^3-RNH-SO_2R'-X_2) gave a new class of acridine compounds, which on alkylation gave the acridinium salts. Similarly, substitution of a 6-chlorocarbonyl phenanthridine for the acridine in this scheme gives rise to a new class of phenanthridinium salts. These acridinium and phenanthridinium salts are useful for chemiluminescent labeling of proteins, nucleic acids and small molecules used in diagnostic testing.

Several acridinium sulfonylamides were prepared which have specific activity and stability suitable for use in diagnostic testing, particularly in CLIA. The synthesis of these compounds allows for the introduction of a variety of functional groups (X^1, X^2, X^3) which may be used in antibody labeling. In addition, the kinetics of the chemiluminescent reaction may be controlled by the choice of the substitutents (R, R′) on the sulfonamide leaving group.

The compounds were evaluated for their efficiency by diluting 20 µl of a 10^{-9} M solution of the compound with 300 µl of 0.1N HCL, then adding 150 µl of 0.03% H_2O_2 in 0.2 N NaOH to trigger the chemiluminescence. Chemiluminescence was measured on a photon-counting luminometer. The light output was recorded as total photon counts, from which the efficiency of each compound was calculated as counts/mole. These are relative numbers, since the efficiency of the photon counting was instrument-dependent. Direct comparisons of compounds were carried out on the same instrument. The results are presented in Table I which the structures may be identified by the formula



wherein R''-X^1 is CH_3, and R'-X^2 and R-X^3 are as indicated in Table 1, chemiluminscent output is abbreviated "CTS/MOLE," the time required for total light output is abbreviated "INT. TIME" and the time required to reach peak light output is abbr viated "PEAK CTS."

TABLE 1

$R'-X^2$	$R-X^3$	CTS/MOLE ($X\ 10^{-18}$)	INT. TIME(SEC)	PEAK CTS (SEC)
CF_3	C_6H_5	12	1	0.22
$o-NO_2C_6H_4$	C_6H_5	10	2	0.23
$p-Br-C_6H_4$	C_6H_5	9	2	0.24
CF_3	$i-C_3H_7$	15	2	0.25
$p-NO_2-C_6H_4$	$n-C_4H_9$	8	2	0.25
$o-NO_2-C_6H_4$	$i-C_3H_7$	11	2	0.25
$p-CH_3C_6H_4$	C_6H_5	9	2	0.27
$o-NO_2C_6H_4$	$n-C_4H_9$	6	2	0.29
$2,4-di-NO_2C_6H_3$	$n-C_4-H_9$	5	2	0.32
$p-BrC_6H_4$	$n-C_4H_9$	7	3	0.44
$p-BrC_6H_4$	$i-C_3H_7$	12	6	0.44
$p-CH_3C_6H_4$	$n-C_4H_9$	5	6	0.98
$p-CH_3C_6H_4$	$i-C_3H_7$	8.3	10	0.96
$2,4,6-(C_3H_7)_3C_6H_2$	$n-C_4H_9$	14	20	4.08
$2,4,6-(CH_3)_3C_6H_2$	$n-C_4H_9$	5	50	11.6
CF_3	$C_6H_4CHCO_2$ benzyl	4	2	--

All of the tested compounds were efficient (5-20 x 10^{18} counts/mole). The specific activity was insensitive to the nature of the R and R′ groups at locations indicated above; however, the time required to reach peak light output and the time required for total light output differed by a factor of 50 between the fastest and slowest compounds. Electron withdrawing groups in R and R′ increas d the reaction rate while bulkyl, electfon-donating groups decreased the reaction rate. Although ch miluminscent compounds according to the present invention which have a chemiluminescent lifetime of 2-10 seconds are preferred for immunoassays, compounds having shorter lifetime may be useful as a source of intense, pulsed light, and compounds having a longer lifetime may be us ful as "cold light" sources.

The stability of compounds prepared according to the present invention was assessed in several ways. First, the compounds were diluted to sub-nanomolar solutions in aqueous buffer at pH 5-7. The solutions were incubated at room temperature and at 45 • C, while the decrease in chemiluminescence was monitored over time. This provided qualitative results whereby the relative stability of the compounds was determined. Anomalous results due to non-specific adsorption of the compounds on the incubation container were minimized by the addition of detergents, protein, and the like. Unambiguous, quantitative results were obtained by monitoring millimolar solutions of the compounds by reverse phase high performance liquid chromatography ("HPLC"). The stability of these compounds was affected by R and R' in the same way as were the kinetics of the chemiluminescence reaction, i.e. electron withdrawing groups destabilized and bulky electron donating groups stabilized the compounds.

Although other techniques may be employed to label antibodies, the NHS activation method is presently preferred. Other materials which function well according to the present invention include polyclonal antibodies, monoclonal antibodies, Fab antibody fragments, all of which are hereinafter included in the general term "antibody," haptens, antigens, nucleic acid probes, and non-antibody binding proteins capable of binding complementary small molecular weight analytes (for example, folate binding protein, which binds folic acid, and intrinsic factor, which binds Vitamin B_{12}). Antibody conjugates retain more than 80% chemiluminescence after being heated at 45 • C for four weeks.

A solid phase sandwich immunoassay system for assaying hepatitis B surface antigen ("HBsAg") (Abbott Laboratories, Abbott Park, Illinois) was employed to compare CLIA according to the present invention with RIA. The type of antibody-coated bead, diluent, incubation conditions, washing condition and antibody preparation were the same except that the antibody was labeled with ^{125}I by the chloramine T method for RIA and labeled with NHS-activated N-sulfonyl-9-acridinium carboxamide for CLIA.

A solid phase sandwich immunoassay for human thyroid stimulating hormone (hTSH) was used to compare CLIA with EI (Abbott Laboratories, Abbott Park, Illinois). The EIA employed a horseradish peroxidase ("HRPO")-labelled antibody while the CLIA used an NHS-activated N-sulfonyl-9-acridinium carboxamide.

The present invention is more specifically described in the following examples. In Example 1, the preparation of sulfonamides which are useful in constructing compounds according to the present invention is set forth. Example 2 includes a description of the preparation of N-sulfonyl-9-acridinecarboxamides according to the present invention. In Example 3, the preparation of 10-methyl N-sulfonyl-acridinium carboxamides is described. Examples 4-6 contain descriptions of syntheses of p-toluenesulfonyl (tosyl) compounds according to the present invention. In Example 7, the preparation of acridinecarboxamides is illustrated.

Example 8-10 contain methods for synthesis of some acridinium carboxamides and products thereof according to the present invention. In Example 11, an evaluation of the chemiluminescence of N-sulfonylacridinium carboxamide compounds according to the present invention is provided. Example 12 includes a report of a stability test of an acridinium carboxamide according to the present invention. In Example 13, the temperature and pH stability of two acridinium carboxamides according to the present invention is compared to the temperature and pH stability of an acridiniumcarboxylate. Example 14 is a description of a method for conjugating an antibody, specifically an immunoglobin G ("IgG") antibody, with a compound according to the present invention. The results of a heat stability study of a conjugate according to Example 14 are presented in Example 15. Example 16 includes a description of the preparation of anti-HBsAg acridinium-labeled conjugate as well as a comparison of the sensitivity observed in CLIA and RIA assays employing those conjugates. In Example 18, the synthesis of a phenanthridinium compound according to the present invention is described. Example 17 describes an anti-hTSH acridinium-labeled conjugate along with a comparison to an EIA system.

Example 1

General Method for Preparation Of Sulfonamides

Amine starting materials for compounds 1-13 and 17-21 are available from Aldrich Chemical Co., Milwaukee, Wisconsin. For compounds 14-16 and 22-25, the appropriate aminocarboxylic acid (as obtained from Aldrich Chemical Co., Milwaukee, Wisconsin) was esterified according to standard, published procedures to provide the starting materials.

In order to prepare a sulfonamide according to the present invention, the corresponding amine (200 mole perc nt) was dissolved in anhydrous methylene chloride, and was treated dropwise at 0 • C with a solution (100 mole percent) of the sulfonyl chloride or anhydride. The solution was poured into anhydrous

ether (5 volumes), washed with 1.4 M H_3PO_4 (25 ml) and then brin (25 ml), and dried over $MgSO_4$. After filtering and evaporating, crude sulfonamides were crystallized from an appropriate solvent.

The following sulfonamides were prepared in this manner. In the description accompanying the name of each compound, the abbreviation "MS" identifies peaks, such as the base peak ("M^+") in the mass spectrum at a location (i.e., at an m/e) specified by the symbol "@". A melting point ("M_p") or an indication that the material is a liquid at room temperature (e.g. "oil") or decomposes before melting ("decomp.") may be provided. Each compound is identified by a "compound number" (1-25 in this Example) followed by an "identifying number" (e.g. 13513-227) and a chemical name.

1. 13513-227 N-Phenyl-p-toluenesulfonamide
   MS M^+ @ 247
   M_p 100-102 °C
2. 13513-228 N-Phenyl-p-bromobenzenesulfonamide
   MS M^+ @ 311
   M_p 115-117 °C
3. 13513-229 N-Phenyl-o-nitrobenzenesulfonamide
   MS M^+ @ 278
   M_p 112-113 °C
4. 13513-231 N-Phenyl-p-nitrobenzenesulfonamide
   MS M^+ @ 278
   M_p 168-170 °C
5. 13513-232 N-Phenyl-2,4-dinitrobenzenesulfonamide.
   MS M^+ @ 323
   M_p 110-113 °C
6. 13513-233 N-Phenyl-trifluoromethanesulfonamide
   MS M^+ @ 225
   M_p 65-67 °C
7. 13514-001 N-Isopropyl-p-toluenesulfonamide
   MS M^+ @ 213
   M_p 50-51 °C
8. 13514-002 N-Isopropyl-p-bromobenzenesulfonamide
   MS M^+ @ 277
   M_p 95-96 °C
9. 13514-003 N-Isopropyl-o-nitrobenzenesulfonamide
   MS M^+ @ 244
   M_p 119-120 °C
10. 13514-004 N-Isopropyltrifluoromethanesulfonamide
    MS (M - 1) @ 190
    oil
11. 13514-006 N-Isopropyl-p-nitrobenzenesulfonamide
    MS M^+ @ 244
    M_p 113-114 °C
12. 13514-025 N-Butyl-2,4,6-trimethylbenzenesulfonamide
    MS M^+ @ 255
    M_p 45 °C
13. 13514-026 N-Butyl-2,4,6,-trisopropylbenzenesulfonamide
    MS M^+ @ 339
    M_p 104 °C
14. 13514-032 Benzyl 6-(N-tosylamino)hexanoate
    MS M^+ @ 375
    oil
15. 13514-057 t-Butyl N-tosyl-β-alanine
    MS M^+ @ 242 (M - 57)
    oil
16. 13514-058 Benzyl 5-(N-tosylamino)-pentanoate
    MS M^+ @ 361
    oil
17. 13513-170 N-Butyl-p-toluenesulfonamide,
    MS M^+ @ 227

M_p 42-44 • C

18. 13513-173 N-Butyl-p-bromobenzenesulfonamide,
MS M^+ @ 241
M_p 53-54 • C

19. 13513-172 N-Butyl-o-nitrobenzenesulfonamide,
MS M^+ @ 258
M_p 58-60 • C

20. 13513-174 N-Butyl-p-nitrobenzenesulfonamide
MS M^+ @ 258
M_p 80-81 • C

21. 13513-213 N-Butyl-2,4-dinitrobenzene sulfonamide,
MS M^+ @ 304
M_p 60-62 • C

22. 13513-085 Benzyl 6-(N-trifluoromethyl-sulfonylamino)-hexanoate
oil

23. 13513-083 Benzyl N-(trifluoromethylsulfonyl)-4-(carboxymethyl) aniline

24. 14973-1A Benzyl N-(5-carboxypentyl)-p-bromobenzenesulfonamide
MS M^+ @ 439
M_p 52-56 • C

25. 14973-37A Benzyl N-(5-carboxypentyl)-p-nitrobenzenesulfonamide
MS M^+ @ 406
M_p 86-88 • C

Example 2

Preparation of N-sulfonyl-9-acridinecarboxamides

Freshly sublimed potassium tert-butoxide (200 mole percent) and tri-n-butylbenzylammonium bromide (1 mole percent) were suspended in toluene under nitrogen. A selected sulfonamide (200 mole percent) was added, the mixture was stirred for 10-30 minutes before evaporating to dryness and the dried material resuspended in the solvent. [Alternatively, the phase transfer catalyst may be omitted and an appropriate anion may be generated in tetrahydrofuran.] After the addition of 9-chlorocarbonylacridine hydrochloride (100 mole percent), the reaction mixture was stirred for 3 to 14 hours at room temperature until no further change was noted by thin-layer chromatography ("TLC"). The reaction solution was diluted with ethyl ether (10 volumes) and washed with brine (25 ml). After drying over $MgSO_4$, filtering and evaporating, the crude product was chromatographed (on a Chromatotron™ chromatograph [available from Harrison Research, Palo Alto, California] using a 2 mm silica rotor and employing an ethylacetate/hexane gradient). The fractions containing the product were collected, evaporated and crystallized from ether/heptane (i.e., the fractions were dissolved in ether followed by the addition of heptane until the mixture became cloudy).

The following compounds were prepared from starting materials as indicated in brackets wherein starting materials prepared herein are identified by the number associated with them in Example 1 or in this example, and wherein a commercial source is provided in brackets for each identified starting material not synthesized herein. All other notations are explained in Example 1.

26. 13513-234 N-Phenyl-N-p-toiuenesulfonyl-9-acridinecarboxamide
[compound 1]
MS M^+ @ 452
M_p 200 • C

27. 13513-236 N-Phenyl-N-p-bromobenzene-sulfonyl 9-acridinecarboxamide
[compound 2]
MS M^+ @ 516
M_p 218-219 • C

28. 13513-240 N-Phenyl-N-o-nitrobenzene-sulfonyl 9-acridinecarboxamide
[compound 3]
MS M^+ @ 483
M_p 197-200 • C

29. 13513-242 N-Phenyl-N-p-nitrobenzenesulfonyl-9-acridinecarboxamide
[compound 4]
MS M^+ @ 483

30. 13513-243 N-Phenyl-N-trifluoromethanesulfonyl-9-acridinecarboxamide
[compound 6]
MS M^+ @ 430
M_p 162 • C

31. 13514-007 N-Isopropyl-N-p-toluenesulfonyl-9-acridinecarboxamide
[compound 7]
MS M^+ @ 418
M_p 163-164 • C

32. 13514-009 N-Isopropropyl-N-p-bromobenzenesulfonyl-9-acridinecarboxamide
[compound 8]
MS M^+ @ 482
M_p 205 • C

33. 13514-012 N-Isopropyl-N-o-nitrobenzenesulfonyl-9-acridinecarboxamide
[compound 9]
MS M^+ @ 449
M_p 215 • C

34. 13514-001 N-Isopropyl-N-trifluoromethane sulfonyl-9-acridinecarboxamide
[compound 10]
MS M^+ @ 396

35. 13514-028 N-Butyl-N-2,4,6,-trimethylbenzenesulfonyl-9-acridinecarboxamide
[compound 12]
MS M^+ @ 460
M_p 88-90 • C

36. 13514-031 N-Butyl-2,4,6-triisopropylbenzenesulfonyl-9-acridinecarboxamide
[compound 13]
MS M^+ @ 544

37. 13514-042 N-tosyl-N-(5-carboxypentyl)-9-acridinecarboxamide, benzyl ester
[compound 14]
MS M^+ @ 550
oil

38. 13514-062 N-tosyl-N-(4-carboxybutyl)-9-acridinecarboxamide, benzyl ester
[compound 16]
MS M^+ @566

39. 13514-069 N-tosyl-N-(2-carboxyethyl)-9-acridinecarboxamide, t-butyl ester
[compound 15]
MS M^+ 504
M_p 157-158 • C

40. 13513-186 N-Butyl-N-p-toluenesulfonyl-9-acridinecarboxamide
[compound 17]
MS M^+ @ 432
M_p 122-123 • C

41. 13513-191 N-Butyl-N-o-nitrophenylsulfonyl -9-acridinecarboxamide
[compound 19]
MS M^+ @ 463
M_p 170 • C

42. 13513-195 N-Butyl-N-p-nitrophenylsulfonyl-9-acridinecarboxamide
[compound 20]
MS M^+ 463
M_p 210 • C

43. 13513-218 N-Butyl-N-(2,4-dinitrophenylsulfonyl) -9-acridinecarboxamide
[compound 21]
MS M^+ @ 508
M_p 95 • C

44. 14973-9C N-(5-carboxypentyl)-N-p-bromobenzenesulfonyl-9-acridinecarboxamide, benzyl ester
[compound 24]
MS (M + H) @ 645

45. 14973-40C N-(5-carboxypentyl)-N-p-nitrobenzenesulfonyl-9-acridinecarboxamide, benzyl ester
[compound 25]

MS (M + H) @ 645

46. 14973-88A N-p-Toluenesulfonyl-9-acridinecarboxamide [p-toluene sulfonamide (Aldrich)]
M_p 276 • C

47. 14973-21C N-Allyl-N-p-toluenesulfonyl-9-acridinecarboxamide
[compound 46]
M_p 136-138 • C

48. 13513-202 N-Butyl-N-p-bromobenzenesulfonyl-9-acridinecarboxamide
MS M^+ @ 496/498
M_p 148-149 • C

Example 3

Preparation of 10-Methyl N-sulfonylacridinium carboxamides

Methylation of N-sulfonylacridine carboxamides was performed according to the following procedure. Each acridine sulfonylamide was dissolved in anydrous methylene chloride. Anhydrous Na_2CO_3 (5 X weight of the sulfonimide) was added followed by methyl triflate (20 X weight of the sulfonimide). The suspension was stirred under nitrogen for 14-48 hours at room temperature to 40 • C. The reaction was monitored by TLC (reverse phase). The product was obtained after filtration and evaporation of the solvent and of excess methyl triflate. Purification was achieved by triturating the solid residue with hot benzene or by reverse phase HPLC.

The following compounds were prepared, and they are described according to the numerals, symbols and abbreviations which are explained in Example 1 or in Example 2.

49. 13513-246 10-Methyl-N-phenyl-N-p-toluenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate
[compound 26]
MS M^+ @ 467
M_p 210-24 • C (decomp.)

50. 13513-247 10-Methyl-N-phenyl-N-p-bromobenzenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate
[compound 27]
MS M^+ @ 531, 533
M_p 240 • C (decomp.)

51. 13513-248 10-Methyl-N-phenyl-o-nitrobenzenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate
[compound 28]
MS M^+ @ 490
M_p 248-50 • C (decomp.)

52. 13513-249 10-Methyl-N-phenyl-N-trifluoromethanesufonyl-9-acridinium carboxamide trifluoromethanesulfonate
[compound 30]
MS M^+ @ 445

53. 13513-250 10-Methyl-N-phenyl-p-nitrobenzenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate
[compound 29]
MS M^+ @ 484

54. 13514-013 10-Methyl-N-isopropyl-N-p-toluenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate
[compound 31]
MS M^+ @ 433
M_p 214 • C

55. 13514-014 10-Methyl-N-isopropyl-N-p-bromobenzenesulfonyl-9-acridinium carboxamide trifluoromethan sulfonate
[compound 32]
MS M^+ @ 497/499
M_p 200 • C (decomp)

56. 13514-018 10-M thyl-N-isopropyl-N-o-nitrobenzenesulfonyl-9-acridinium carboxamide trifluoromethan sulfonate

[compound 33]
MS M^+ @ 464

57. 13514-021 10-Methyl-N-isopropyl-N-trifluoromethanesulfonyl-9-acridinium carboxamide trifluormethanesulfonate
[compound 34]
MS M^+ @ 411

58. 13514-037 10-Methyl-N-butyl-N-(2,4,6-trimethylbenzenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate
[compound 35]
MS M^+ @ 475
M_p 227 • C (decomp.)

59. 13514-038 10-Methyl-N-butyl-N-(2,4,6 triisopropylbenzenesulfonyl-9--acridinium carboxamide trifluoromethanesulfonate
[compound 36]
MS M^+ @ 559
M_p 231 • C (decomp.)

60. 13514-044 10-methyl-N-tosyl-N-(5-carboxypentyl)-9 -acridinium carboxamide trifluoromethanesulfonate, benzyl ester
[compound 37]

61. 13514-079 10-methyl-N-tosyl-N-(2-carboxyethyl)-9-acridinium carboxamide trifluoromethanesulfonate , t-butyl ester
[compound 39]
MS M^+ @ 519
M_p 207 • C (decomp.)

62. 13513-211 10-Methyl-N-butyl-N-p-toluenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate.
[compound 40]
MS M^+ @ 447

63. 13513-212 10-Methyl-N-butyl-N-p-bromobenzenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate
[compound 48]
MS M^+ @ 511
M_p 126 • C

64. 13513-215 10-Methyl-N-butyl-N-o-nitrophenylsulfonyl-9-acridinium carboxamide trifluoromethanesulfonate
[compound 41]
MS M^+ @ 478
M_p 232-234 • C

65. 13513-216 10-Methyl-N-butyl-N-p-nitrophenysulfonyl-9-acridinium carboxamide trifluoromethanesulfonate
[compound 42]
MS M^+ @ 478
M_p 201 • C

66. 13513-230 10-Methyl-N-butyl-N-(2-4 dinitrophenylsulfonyl)-9-acridinium carboxamide trifluoromethanesulfonate
[compound 43]
MS M^+ @ 523
M_p 215-220 • C

67. 14973-31B 10-Methyl-N-allyl-N-p-toluenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate
[compound 47]
MS M + 2 @ 433

68. 14973-47A 10-methyl-N-(5-carboxypentyl)-N-p-nitrobenzenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate, benzyl ester
[compound 45]
MS M^+ @ 626
M_p 139-141 • C

69. 14973-90A 10-Methyl-N-methyl-N-p-toluenesulfonyl-9-acridinium carboxamide trifluoromethane-

sulfonate
[compound 46]
MS M^+ @ 405

70. 14973-25A 10-methyl-N-(5-carboxypentyl)-N-(o-bromobenzenesulfonyl)-9-acridinium carboxamide, benzyl ester
[compound 44]

Example 4

Synthesis of 10-methyl-N-tosyl-N-(6-hexanoyl-N-hydroxysuccinimido)-9-acridinium carboxamide trifluoromethanesulfonate

Compound 37 (450 mg, 0.78 mmoles) was treated with 6 ml of 31% HBr in acetic acid at 50 °C for 2 hours under N_2. The solution was poured into 30 ml of water and cooled. Carboxylic acid compound 71, 13514-045 [N-tosyl-N-(5-carboxypentyl)-9-acridinecarboxamide] was separated by filtration.

Compound 71 (100 mg., 0.2 mmol) was dissolved in dry methylene chloride (5 ml) and treated with N-hydroxysuccinimide (23 mg, 0.2 mmol) and dicyclohexylcarbodiimide (41 mg) under N_2 for 12 hours. After reacting, the solution was filtered and then evaporated to dryness to yield an active ester, compound 72, 13514-052 [N-tosyl-N-(6-hexanoyl-N-hydroxysuccinimido)-9-acridinecarboxamide].

Compound 72 was methylated as in Example 3 to give compound 73. Compounds 71, 72, and 73 are described below using the numerals, symbols and abbreviations which are explained in Example 1.

71. 13514-045 N-Tosyl-N-(5-carboxypentyl)-9-acridinecarboxamide
[compound 37]
MS M^+ @ 240
M_p 150-152 °C

72. 13514-052 N-Tosyl-N-(6-hexanoyl-N-hydroxysuccinimido)-9-acridinecarboxamide
[compound 71]
MS M^+ @ 588

73. 13514-054 10-Methyl-N-tosyl-N-(6-hexanoyl-N-hydroxysuccinimido)-9-acridinumcarboxamide trifluoromethanesulfonate
[compound 72]

Example 5

Synthesis of 10-Methyl-N-tosyl-N-(5-pentanoyl-N-hydroxysuccininimido)-9-acridinium carboxamide trifluoromethanesulfonate

Compound 38, 13514-062, was treated as in Example 4 and yielded compound 74, 13514-065 [N-tosyl-N-(4-carboxybutyl)-9-acridinecarboxamide].

Compound 74 was coupled to N-hydroxysuccinimide, as in Example 4, to give compound 75, 13514-067, N-tosyl-N-(5-pentanoyl-N-hydroxysuccinimido)-9-acridinecarboxamide. This compound was methylated as in Example 3 to give compound 76, 13514-078 [10-methyl N-tosyl-N-(5-pentanoyl-N-hydroxysuccinimide)-9-acridinium carboxamide trifluoromethanesulfonate].

Compounds 74, 75 and 76 are described using the numerals, symbols and abbreviations which are explained in Example 1.

74. 13514-065 N-Tosyl-N-(4-carboxybutyl)-9-acridinecarboxamide
MS M^+ @ 476
M_p 152-155 °C

75. 13514-067 N-Tosyl-(5-pentanoyl N-hydroxy succinimido)-9-acridinecarboxamide
[compound 74]
MS M^+ @ 573

76. 13514-078 10-Methyl-N-tosyl-N-(5-pentanoyl-N-hydroxysuccinimido)-9-acridinium carboxamide trifluoromethan sulfonate
[compound 75]

Example 6

Synthesis of 10-methyl-N-tosyl-N-(2-carboxyethyl-9-acridinium carboxamide trifluoromethanesulfonate

Compound 61, 13514-079 (50 mg, 0.072 mmol) was dissolved in 2 ml of trifluoroacetic acid ["TFA"] at 0 °C under N_2. After stirring for 15 minutes, the TFA was evaporated and the residue was recrystallized from methanol/ether (i.e., the residue was dissolved in methanol, adding ether until cloudy). Alternatively, compound 61, was refluxed in 1 N HCl for 3 hours. The aqueous solution was evaporated to dryness to leave a residue, and the residue was purified by preparative reverse phase HPLC. Compound 77, 13514-081 [10-methyl N-tosyl-N-(2-carboxyethyl)-9-acridinium carboxamide] resulted from either approach. Compound 77 is described using the numerals, symbols and abbreviations which are explained in Example 1.

77. 13514-081 10-Methyl-N-tosyl-N-(2-carboxyethyl)-9-acridinium carboxamide trifluoromethanesulfonate
[compound 61]
MS (M + 14) @ 477; M^+ @ 463
M_p 227 °C (decomp.)

Example 7

Preparation of Acridinecarboxamides

An amine (110 mole percent) and triethylamine (220 mole percent) were dissolved in methylene chloride. One hundred mole percent of 9-chlorocarbonyl acridine was added dropwise as a solution in methylene chloride. The reaction was stirred under N_2 for 3 hours. The solution was filtered through silica gel and the filtrate was evaporated to leave a residue. The residue was then recrystallized from an appropriate solvent (isopropyl ether for compound 78 and ethyl ether for compound 79).

The following amides were prepared, and are described using the numerals, symbols and abbreviations which are explained in Example 1.

78. 14973-15A N-Allyl-9-acridinecarboxamide [Allyl amine (Aldrich)]
MS M^+ @ 262
M_p 192 °C

79. 14973-6A Benzyl N-(5-carboxypentyl)-9-acridinecarboxamide [6-Amino caproic acid (Aldrich)]
MS M^+ @ 458
M_p 86 °C

Example 8

Synthesis of Acridinium carboxamides

An ester (either compound 44 or compound 68) was added to a 1 N HCl solution and refluxed for 3-4 hours. Upon cooling, the suspension was either filtered and the product collected, or the suspension was extracted with a chloroform:isopropanol (3:2) mixture, which provided the desired product (compound 80 or 81, respectively) on evaporation. Compounds 80 and 81 are described using the numerals, symbols and abbreviations which are explained in Example 1.

80. 14379-27A 10-Methyl-N-(5-carboxypentyl)-N-p-bromobenzenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate
[compound 44]
MS M^+ @ 569, 571
M_p 148-150 °C

81. 14973-51A 10-Methyl-N-(5-carboxypentyl)-N-p-nitrobenzenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate
[compound 68]
MS M^+ @ 536

Example 9

Synthesis of 10-(3-sulfopropyl)-N-tosyl-N-(2-carboxyethyl)-9-acridinium carboxamide

Propane sultone (260 mole percent) was heated with t-butyl N-tosyl-N-(2-carboxyethyl)-9-acridinecarboxamide (compound 39, 13514-069) at 110 -120 • C for 2 hours. After cooling, the solid mass was taken up in methanol and filtered. The filtrate was evaporated to dryness and the residue triturated with benzene to remove un-quaternized material.

The crude product compound was treated with trifluoracetic acid at 0 • C then allowed to warm to 25 • C over a period of 15 minutes. The residue obtained upon evaporation was purified chromatographically on preparative thick-layer chromatography plates (C-18 PLKC 18F, 20 x 20 cm, 1000M, as available from Whatman, Clifton, New Jersey), eluted with 70 parts methanol/30 parts 0.5% aqueous acetic acid, and further purified by ion exchange on Cellex-D™ resin [BioRad Laboratories, Richmond, California] using 8% formic acid to elute the product, compound 82, which is described below using the numerals, symbols and abbreviations which are explained in Example 1.

82. 14496-243 10-(3-sulfopropyl)-N-tosyl-N-(2-carboxyethyl)-9-acridinium carboxamide
[compound 39]
MS M^+ @ 572

Example 10

Synthesis of 10-(3-sulfopropyl)-N-tosyl-N-(3-sulfopropyl)-9-acridinium carboxamide

Fifty milligrams of N-tosyl-9-acridinecarboxamide (compound 46, 14973-88A) were heated at 140-150 • C under argon in a sealed tube with 500 mg of propane sultone for 3 hours. After cooling, excess propane sultone was removed by trituration with benzene (5 ml X 3). The crude product was purified by anion exchange chromatography using BioRad AG-1-X4 formate form [BioRad Laboratory, Richmond, California], eluted with a gradient of aqueous formic acid. The product, compound 83, is described below using the numerals, symbols and abbreviations explained in Example 1.

83. 30253-020 10-(3-Sulfopropyl)-N-tosyl-N-(3-sulfopropyl)-9-acridinium carboxamide.
[compound 46]
MS M + H @ 621.

Example 11

Evaluation of N-sulfonylacridinium carboxamide Chemiluminescence

Acridinium compounds to be tested for chemiluminescence were dissolved in dimethyl formamide ("DMF") and then diluted with 0.05 M sodium citrate (pH 5.0) or 0.05 M sodium phosphate (pH 7.0) buffer to give solutions of about 3 X 10^{-9} M. Twenty microliters of each buffered solution was diluted with 300 µl of 0.1 N HCl and chemiluminscence was triggered with 150 µl of 0.03% H_2O_2 in 0.2 N NaOH.

The light generated was recorded on a photon counter luminometer over a 10 second interval except where a longer interval is indicated in Table 2. The specific activity of each compound is provided in the form of counts/mole in Table 2.

TABLE 2

Compound No.	Identifying No.	Counts/Mole
49	13513-246	9.4×10^{18}
50	13513-247	9×10^{18}
51	13513-248	1×10^{19}
50	13513-249	1.2×10^{19}
53	13513-250	1×10^{19}
54	13514-013	8.3×10^{18}
55	13514-014	1.25×10^{19}
56	13514-018	1.1×10^{19}
57	13514-021	1.5×10^{19}
58	13514-037	5.2×10^{18} (50 secs)
59	13514-038	1.4×10^{19} (20 secs)
62	13513-211	5×10^{18}
63	13513-212	7×10^{18}
64	13513-215	6.1×10^{18}
65	13513-216	8×10^{18}
66	13513-230	5×10^{18}

Example 12

Stability Test of Compound 62 (13513-211)

Compound 62 (2 mg) was dissolved in 1 ml of methanol. Fifty microliters of this solution were added to each of the following buffers:

1) 500 microliters of 0.05 M sodium phosphate, pH 5.0
2) 500 microliters of 0.05 M sodium phosphate, pH 5.5
3) 500 microliters of 0.05 M sodium phosphate, pH 6.0
4) 500 microliters of 0.05 M sodium phosphate, pH 6.5
5) 500 microliters of 0.05 M sodium phosphate, pH 7.0.

Each solution was analyzed on a Perkin-Elmer Series 4 HPLC using a reverse phase column (C-18 μ Bondapak, 3.9 mm x 30 cm, available from Waters Associates, Milford, Massachusetts). The elution was done with 75% methanol and 25% 5 mM pentanesulfonic acid in 1% aqueous acetic acid at a flow rate of 1 ml/min. The effluent was monitored at 254 nm.

After 4 weeks at room temperature, the solutions at pH 5.0, pH 5.5 and pH 6.0 showed no sign of decomposition, while at pH 6.5 and at pH 7.0, 20% and 70% decomposition were seen, respectively.

Example 13

Comparison of Temperature and pH Stabilities of Acridinium Compounds in Buffer at pH 7.2

Three different acridinium compounds, compound 62, 13513-211, a compound identified by the number 13514-020 [4-(carbobenzyloxymethyl)-phenyl-10-methyl-9-acridinium carboxylate trifluoromethanesulfonate] as prepared as in Weeks, et al., Clin. Chem., 29, 1474-79 (1983), and compound 83, 30253-020, were compared for temperature and pH stability. The comparison was carried out in methanol or water at a concentration of 1.0 mg/ml (which is approximately equivalent to 1.6×10^{-3} M). Each of the samples was diluted 1:100 in an acid solution containing one part of 0.1 N HCl plus one part phosphate-buffered saline ("PBS") pH 6.8 with 0.01% Tween 20® (available from Sigma Chemical Company, St. Louis, Missouri). The final pH of the diluent solution were about 1.5. The molarity of each of these solutions was 1.6×10^{-5} M.

Each of the solutions was scanned to record a UV-visible absorption spectrum in order to determine molar extinction coefficients and in order to detect any appreciable differences in the absorbance spectra. The UV-visible absorption spectra of these acridinium compounds have the characteristics presented in Table 3.

TABLE 3

Compound No.	Identifying No.	Wavelength	Observed Absorbance
62	13513-211	263nm 369nm	1.40 0.286
83	30253-020	263.5nm 370nm	1.42 0.304
	13514-020	262nm 368nm	1.72 0.334
For all three compounds, $\epsilon_{370} \cong 18{,}000$ and $\epsilon_{263} \cong 87{,}000$.			

These spectra indicate that there is very little difference either in UV-visible absorbance or in molar extinction coefficients among these three compounds. In fact, within the limitations of experimental error, few or no spectral differences were observed.

The 1.6×10^{-5} M stock solutions of the three compounds were serially diluted 10-fold in 0.01 M sodium phosphate with 0.05% normal human serum at pH 4.8. They were also serially diluted 10-fold in PBS (pH 7.2) with 0.01% Tween 20®.

Because it is known that, in general, acridinium compounds are more stable at an acid pH, it was assumed that the counts obtained from the samples diluted in pH 4.8 buffer would be representative of the maximum stability with maximum chemiluminescent output. All three compounds were serially diluted 10-fold to a final concentration of 1.6×10^{-10} M. A 10 µl aliquot of each sample was added to 90 µl of 0.05 N HCl. Chemiluminescence was triggered with 200 µl of 0.03% H_2O_2 in 0.25 N NaOH and counts were monitored on a luminometer for 6 seconds with results as presented in Table 4. Results are presented in Table 4 for each of three runs.

TABLE 4

Compound No.	Identifying No.	Counts/6 Seconds
62	13513-211	92,669 91,241 91,995
83	30253-020	138,791 141,962 145,133
	13514-020	59,438 59,443 59,449

Within experimental error, chemiluminescent output on the luminometer did not differ among the compounds, as indicated in Table 5.

TABLE 5

Chemiluminescent Output at pH 4.8		
Compound No.	Identifying No.	Counts/Mole
62	13513-211	5.7×10^{19}
83	30253-020	8.7×10^{19}
	13514-020	3.7×10^{19}

When 10 µl of these same compounds were diluted to 1.6×10^{-10}M in 90 µl PBS buffer (pH 7.2) with 0.01% Tween 20® and not acidified prior to running chemiluminescence output determinations as above, the results were somewhat different, especially for the acridinium carboxylate compound 13514-020, as shown in Table 6. Results are presented in Table 6 for each of three runs.

TABLE 6

Chemiluminescent Output at pH 7.2

Compound No.	Identifying No.	Counts/6 Seconds
62	13513-211	88,633
		89,135
		90,394
83	30253-020	133,560
		137,929
		142,299
	13514-020	8,185
		7,274
		6,363

The compound identified by the number 13514-020 produced only 4.4 X 10^{18} counts/mole in pH 7.2 buffer, almost an order of magnitude fewer counts than it produced at pH 4.8. This may be due to pseudobase formation by a large proportion of the molecules at the more alkaline pH, the pseudobase being substantially less chemiluminescent than the corresponding positively charged acridinium compound.

The N-sulfonylacridinium carboxamide compounds showed only a very small drop in counts when incubated at pH 7.2. This suggests that they do not undergo pseudobase formation to any appreciable degree, at least at this pH.

The dilution series of all three of the acridinium compounds in pH 7.2 buffer were stored overnight at room temperature and then assayed. Both N-sulfonylacridium carboxamide compounds showed virtually no change in chemiluminescence. The phenyl acridiunium carboxylate showed a significant drop after 20 hours at room temperature.

The samples were then placed in an incubator at 45 °C. Every day for the duration of the study they were removed from the incubator, cooled to room temperature, and 10 µl aliquots diluted in 90 µl of PBS buffer (pH 7.2) were assayed for chemiluminescence.

Neither of the N-sulfonylacridinium carboxamides showed any significant difference in chemiluminescent output when diluted either in 0.05 N HCl or in PBS at pH 7.2. However, the acridinium carboxylate 13514-020 exhibited a significantly different chemiluminscent output when diluted in 0.05 N HCl or in PBS buffer at pH 7.2. When diluted in PBS buffer (pH 7.2), the acridinium carboxylate consistently produced at least 10-fold fewer counts than when diluted in 0.05 N HCl.

The 10,N-bis-(3-sulfopropyl) acridinium carboxamide (compound 83, 30253-020) appears to be quite stable at pH 7.2 at 45 °C. After 10 days under such conditions no appreciable loss of chemiluminescence was observed. Compound 13513-211 produced 10-fold fewer counts, and the acridinium carboxylate 13514-020 produced 10^3 fewer counts under the same conditions.

Example 14

Preparation of Labeled IgG

Disulfopropyl compound 83, 30253-020, was activated by treatment with phosphorous oxychloride in acetonitrile at 45 °C for 12 hours under argon. The solvent and excess $POCl_3$ were removed in vacuo and

the activated compound was used directly in the labeling reaction.

Thus, 10 mg of rabbit IgG (Sigma Chemical Company, St. Louis, Missouri) was dissolved in 0.1 M sodium phosphate buffer (2 ml, pH 7.0) containing 1% Tween 80®. One ml of this solution was mixed with about 2 mg of the bis-sulfonylchloride. The solution was agitated periodically by sonication and stirring for one hour at room temperature.

An aliquot (0.5 ml) of the reaction solution was chromotographed over Sephadex® G-25 (10 cm X 0.75 cm), as available from Pharmacia, Piscataway, New Jersey, and eluted with 0.1 M phosphate buffer (pH 6.5).

The labeled protein eluted as a weakly green fluorescent band. The labeled protein was further purified by HPLC using a Bio-Sil® TSK-250 column (BioRad, Richmond, California). The resulting conjugate (30253-34) contained 0.8 labels/protein, as determined from the ratio of the absorbance of 370 nm ($\epsilon \cong 10{,}000$, acridinium salt) to the absorbance 280 nm ($\epsilon \cong 210{,}000$, IgG).

Example 15

Heat Stability Studies

The conjugate 30253-34, as synthesized in Example 14, was serially diluted 10-fold in three buffers (0.1 M sodium phosphate, 0.01% Tween 20®, pH 6.3; 0.01 M sodium phosphate, 0.15 M NaCl, 0.01% Tween 20®, pH 6.8; and 0.01 M sodium phosphate, 0.15 M NaCl, 0.01% Tween 20®, pH 7.2) to a concentration of 2×10^{-9} M IgG and 1.6×10^{-9} M acridinium. A dilution series was prepared and initial counts were recorded by taking 10 µl of the sample, diluting with 90 µl of PBS buffer at pH 6.3, pH 6.8, or pH 7.2, and then triggering chemiluminescence with 200 µl of 0.03% H_2O_2 in 0.25 N NaOH. A 100 µl sample of PBS buffer was used as a control for each series.

Counts shown in Table 7 are averages of results for duplicate samples assayed on the day on which the dilution series was prepared. The concentration shown in Table 7 is the concentration of the sample prior to dilution. The amount in parentheses for each entry in Table 7 is the amount of conjugate present in the sample.

TABLE 7

Concentration (Amount)	Counts/6 Seconds
pH 6.3	
buffer (0 moles)	253
2×10^{-10} M (2×10^{-14} moles)	216,054
1×10^{-10} M (1×10^{-14} moles)	100,842
5×10^{-11} M (5×10^{-15} moles)	48,704
2.5×10^{-11} M (2.5×10^{-15} moles)	23,771
1.25×10^{-11} M (1.25×10^{-15} moles)	11,475
6×10^{-12} M (6×10^{-16} moles)	5,866
pH 6.8	
buffer (0 moles)	233
2×10^{-10} M (2×10^{-14} moles)	295,608
1×10^{-10} M (1×10^{-14} moles)	149,725
5×10^{-11} M (5×10^{-15} moles)	76,820
2.5×10^{-11} M (2.5×10^{-15} moles)	38,801
1.25×10^{-11} M (1.25×10^{-15} moles)	18,408
6×10^{-12} M (6×10^{-16} moles)	9,398
pH 7.2	
buffer (0 moles)	726
2×10^{-10} M (2×10^{-14} moles)	309,445
1×10^{-10} M (1×10^{-14} moles)	156,311
5×10^{-11} M (5×10^{-15} moles)	77,238
2.5×10^{-11} M (2.5×10^{-15} moles)	39,879
1.25×10^{-11} M (1.25×10^{-15} moles)	19,925
6×10^{-12} M (6×10^{-16} moles)	10,526

Each dilution series was placed in a warm air incubator at 45°C after an initial reading was taken. A duplicate reading was made on each sample daily and then the readings were averaged.

When the conjugate was stored at pH 6.8 and at 45°C, there was no loss in chemiluminescent activity of the label over a 15 day period of observation, at any dilution. Essentially the same results were observed when the conjugate was stored in PBS buffer at pH 7.2.

Example 16

Comparison of CLIA vs. RIA

A. Preparation of Acridinium-Labeled Anti-HsAg Conjugate. Compound 75 (13514-081, Example 6) (12.5 µmol) was dissolved in 200 µl of DMF, was treated with NHS (dissolved in 50 µl of DMF) and

dicycloh xylcarbodiimide (dissolved in 50 µl of DMF) ("DCC"); and stirred for 12 hours at room temperature. The solution of the activated ester was mixed with mouse monoclonal anti-HBsAg in 0.1 M sodium phosphate buffer (pH 6.3) in a molar ratio of 100:1 at 4 • C for 12 hours.

The conjugate was then dialysed against PBS buffer, pH 6.3, until the absorbance of the dialysate indicated no free label. A UV spectral analysis indicated between 2 to 6 labels/antibody (as determined from a ratio of absorbances as in Example 14).

B. Assay for HBsAg. Either type A_d or type A_y HBsAg (200 µl) was diluted in calf serum and was reacted with an Auszyme™ (Abbott Laboratories, Abbott Park, Illinois) monoclonal antibody bead and 2 X 10^5 of counts of ^{125}I-labeled mouse monoclonal anti-HBsAg antibody (40 µl, in the RIA) or an acridinium-labeled mouse monoclonal anti-HBsAg antibody (40 µl, in the CLIA) in PBS containing 50% calf serum, 10% human serum, 0.05% Tween 20® and 5 mM EDTA (pH 6.3), for three hours at 40 • C. The beads were then washed 6 times in water and counted for their activities. Calf serum was used as a negative control.

In the CLIA, a polystyrene bead with conjugate adsorbed thereto was mixed with 250 µl phosphate, 0.5 mM, pH 5.3, in a glass vial suitable for use in a luminometer. While the sample was in the measuring position, 0.2 ml of 0.03% H_2O_2 in 0.25 N NaOH was then injected into the glass vial. The light emmitted was measured in the luminometer. Reading began 0.012 seconds before initiation of the chemical reaction and continued for 6 seconds.

The results are presented in Table 8.

TABLE 8

Concentration (ng/ml)	CLIA		RIA	
	A_d	A_y	A_d	A_y
1.0	2214	3144	371	400
0.5	1256	2494	236	408
0.25	701	921	221	248
0.125	521	592	173	179
Calf Serum	151	179		
Cut-off	327	376		

Under the stated conditions, the sensitivity for the CLIA was less than 0.125 ng/ml for both the A_d and A_y types of HBsAg. For the RIA the sensitivity was 1.0 ng/ml for both the A_d and A_y types. The cut-off count was 2.1 times that of the negative control.

Table 8 clearly shows that chemiluminescent immunoassays according to the present invention are more sensitive than comparable radioimmunoassays.

Example 17

A comparison of CLIA and EIA

A. Preparation of labeled anti-hTSH (30234-207). Compound 75 (13514-081, Example 6) (2 mg, 4.3 µmoles) in 200 ml of acetonitrile was treated with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (Sigma, St. Louis, Missouri) (10 µmoles) in 100 µl of acetonitrile and N-hydroxysuccinimide (4-9 µmoles) in 100 µl of acetonitrile for 12 hours at 25 • C in the dark.

The active ester was mixed with anti-hTSH in PBS buffer containing 0.5% 3-[(3-cholamidopropyl) dimethylammonio]-1-propane-sulfonate ("CHAPS") at pH 6.5 in a ratio of 50:1 (antibody:active ester). After coupling for 3 hours at 25 • C, the labeled antibody was dialysed against PBS buffer containing 0.5% CHAPS at pH 6.5 until no free label was present in the dialysate by U.V.

Based on the U.V. spectra, the conjugate had an average of 10 labels per antibody.

B. Assay for hTSH. CLIA and EIA were compared using the Abbott hTSH-EIA Kit (Abbott Laboratories, Abbott Park, Illinois) with the exception that for the CLIA, the anti-hTSH acridininium conjugate was used in place of the kit anti-hTSH-HRPO conjugate. Thus, a standard curve was generated by incubating the kit standards with the kit beads at 37 • C for 1 hour, then washing three times. For the CLIA, th conjugate prepared above was diluted 1:5000 with PBS buffer containing 50% calf serum, 1% normal mous serum, 0.05% Tween® 20 and 2 mM EDTA at pH 6.3. One hundred microliters of this solution was incubated with the beads for 1 hour at 37 • C, then washed four times.

The beads were transferred one by one to the reaction vial of a luminometer containing 400 µl of water and reacted with 200 µl of 0.03% H_2O_2 in 0.2 N NaOH. Photon counts were recorded for 6 seconds.

The EIA was carried out according to the instructions in the kit insert on a Quantum II® spectro photometer (Abbott Laboratories, Abbott Park, Illinois)

The results are shown in Table 9.

TABLE 9

Concentration (µIu/ml)	CLIA (counts)	EIA (A_{492})
0	533(SD35.4)	0.012
1	5064	0.062
4	14476	0.176
10	32092	0.397
25	66072	0.828
60	110,984	1.602

Under these conditions the sensitivity of the CLIA was 0.016 µIU/ml (0 standard + 2 SD) while the EIA had a sensitivity of 0.05 µIU/ml.

Example 18

Preparation of 5-methyl-6-[N-tosyl-N-(2-carboxyethyl)]-phenanthridiniumcarboxamide

Phenanthridine-6-carboxylic acid (400 mg, 1.8 mmoles) [prepared by the method of Wittig et al., Justus Liebig's Ann., 577, 1 (1952)], was suspended in methylene chloride (20 ml, distilled from P_2O_5) and cooled to 0 °C under nitrogen. Oxalyl chloride (320 µl, 3.6 mmoles) (Aldrich Chemical Co., Milwaukee, Wisconsin) was added, followed by DMF (5 µl). As the reaction mixture was stirred for one hour at 0 °C and for 30 minutes at 25 °C, all the carboxylic acid dissolved. The solution was evaporated to dryness to give the acid chloride which was used without further purification.

Methyl N-tosyl-β-alanine was prepared from methyl-β-alanine (Aldrich Chemical Company, Milwaukee, Wisconsin) and tosyl chloride (Aldrich Chemical Company, Milwaukee, Wisconsin) according to the procedure of Example 1. Potassium t-butoxide (600 mg, 5.4 mmoles, freshly sublimed) was added to a solution of 1.3g (5.4 mmoles) of methyl N-tosyl-β-alanine in 50 ml of THF. After stirring for 15 minutes and at room temperature and under N_2, the suspension was evaporated to dryness. The potassium salt of methyl N-tosyl-β-alanine, was resuspended in 20 ml of THF, mixed with the acid chloride (in 20 ml of THF), and stirred for 12 hours.

The resulting suspension was poured into 100 ml of ethylacetate, washed with 50 ml of water and washed twice with 25 ml of brine. After drying over $MgSO_4$ and evaporating to dryness, the residue was chromatographed on a Chromatatron™ chromatograph (available from Harrison Research, Palo Alto, California) using a 4 mm silica rotor and employing a 25/75 ethylacetate/hexane gradient. The product (R_f 0.2) was collected, then recrystallized from benzene/hexane (i.e., the product was dissolved in benzene, and hexane was added until cloudy) to give 130 mg of methyl 6-[N-tosyl-N-(2-carboxyethyl)]-phenanthridinecarboxamide, Compound 84, 13514-225.

Compound 84, 13514-225, was methylated according to the procedure in Example 3 to give methyl 5-methyl-6-[N-tosyl-N-(2-carboxyethyl)]-phenanthridiniumcarboxamide, compound 85, 13514-227. Compound 85 was hydrolyzed according to the procedure in Example 8 to provide 5-methyl-6-[N-tosyl-N-(2-carboxyethyl)]-phenanthridiniumcarboxamide, compound 86, 13514-228.

Compounds 84, 85 and 86 are described using the numerals, symbols and abbreviations as explained in Example 1.

84. 13514-225 6-[N-tosyl-N-(2-carboxyethyl)]-phenanthridinecarboxylate, methyl ester
MS M + H @ 463

85. 13514-227 5-methyl-6-[N-tosyl-N-(2-carboxyethyl)]-phenanthridiniumcarboxamide, methyl ester
MS M^+ @ 477
Mp 136°C

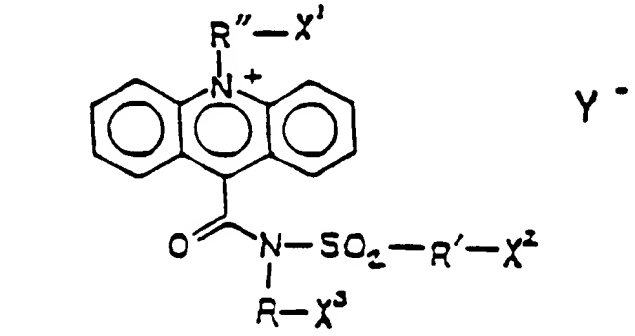
86. 13514-228 5-Methyl-6-[N-tosyl-N-(2-carboxyethyl)]-phenanthridiniumcarboxamide
MS M^+ @ 463

Although the present invention has been described in terms of preferred embodiments, it is understood that modifications and improvements will occur to those skilled in the art.

Claims

Claims for the following Contracting States : BE, CH, DE, FR, GB, GR, IT, LI, LU, NL, SE

1. A chemiluminescent compound selected from compounds identified by the formulae



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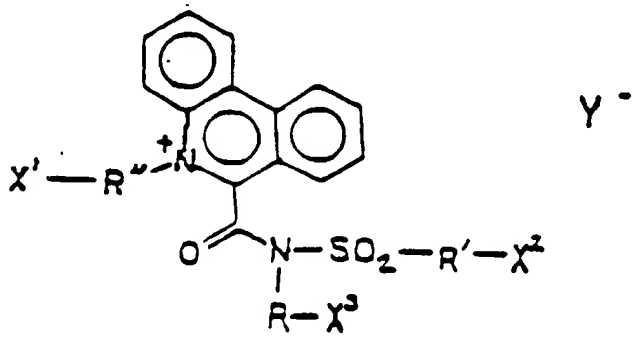
and



II

wherein R, R' and R'' independently comprise a member selected from the group consisting of alkylene, arylene, substituted alkylene, and substituted arylene groups such that:

one or more hydrogens of said member is replaced by an alkyl, aryl, substituted alkyl, substituted aryl, alkoxy, aryloxy, halo, amino, protected amino, substituted amino, hydroxy, protected hydroxy, oxo, thio, imino, mercapto or substituted mercapto group,

or such that one or more carbon atoms of said member is replaced by a heteroatom;

wherein X^1, X^2 and X^3 are independently members of the group consisting of hydrogen, carboxy, carboalkoxyl, carboxamido, carboaryloxy, cyano, carboximido, isocyanato, isothiocyanato, sulfo, sulfonyl halide, carbonyl halide, N-succinimidylcarboxy and N-maleimide groups; or

wherein one of R'-X^2 or R-X^3 can either be a nitro-benzene, provided that the other one is selected from phenyl, iso-propyl, n-butyl or benzyl 5-carboxypentyl, or a dinitro-benzene, provided that the other one is selected from n-butyl and phenyl; and

wherein Y^- is an appropriate counter ion;

with the proviso that R-X^3, R'-X^2 and R''-X^1 may also independently be hydrogen, and

with the further proviso that when in the compounds of formula I in either one of R'-X^2 and R-X^3, X^2 or X^3 is selected from carbopentachlorophenoxy, carbo-p-nitrophenoxy, carboximido, isothiocyanate, N-maleimide and N-succinimidylcarboxy, and the other one of R'-X^2 and R-X^3 is selected from hydrogen, alkyl, aryl or benzyl, or such aryl or benzyl substituted by alkoxy, aryloxy, amino, or hydroxy,

then X^1 is different from H and R''-X^1 is different from H;

and also selected from 10-methyl-N-allyl-N-p-toluenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate.

2. The chemiluminescent compound as recited in claim 1 wherein Y^- is a counter ion selected from the group consisting of sulfate, alkylsulfate, halosulfate, haloborate, haloacetate, halophosphate, phosphate, halide and trifluoromethanesulfonate.

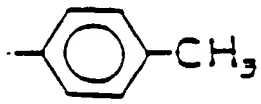
3. The chemiluminescent compound as recited in claim 1 wherein said heteroatom is selected from the group consisting of nitrogen, phosphorus, sulfur and oxygen.

4. The chemiluminescent compound as recited in claim 1 wherein R, R', and R'' independently are of the formula

$$-(CH_2)_n-$$

where n = 0 - 50.

5. The chemiluminescent compound as recited in claim 1 wherein R'' is $-CH_2-$, X^1 is -H, and R'-X^2 is identified by the formula

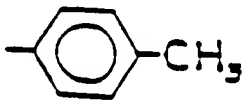


6. The chemiluminescent compound as recited in claim 5 wherein said compound is 10-methyl-N-[2-carboxyethyl]-N-tosyl-9-acridinium carboxamide.

7. The chemiluminescent compound as recited in claim 5 wherein said compound is 10-methyl-N-(4-carboxybutyl)-N-tosyl-9-acridinium carboxamide.

8. The chemiluminescent compound as recited in claim 5 wherein said compound is 10-methyl-N-(5-carboxypentyl)-N-tosyl-9-acridinium carboxamide.

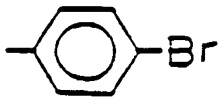
9. The chemiluminescent compound as recited in claim 1 wherein R'' is $-(CH_2)_3-$, X^1 is $-SO_3-$, and R'-X^2 is identified by the formula



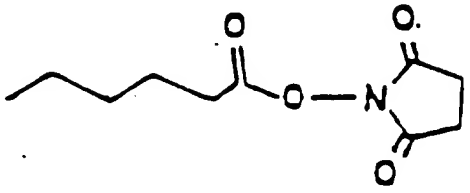
10. The chemiluminescent compound as recited in claim 9 wherein said compound is 10-(3-sulfopropyl)-N-(2-carboxyethyl)-N-tosyl-9-acridinium carboxamide.

11. The chemiluminescent compound as recited in claim 9 wherein said compound is 10-(3-sulfopropyl)-N-(3-sulfopropyl)-N-tosyl-9-acridinium carboxamide.

12. The chemiluminescent compound as recited in claim 1 wherein R'-X^2 is identified by the formula,



and wherein R-X^3 is identified by the formula



13. The chemiluminescent compound as recited in claim 1 wherein said compound is selected from 10-methyl-N-phenyl-N-tosyl-9-acridinium carboxamide trifluoromethanesulfonate, 10-methyl-N-phenyl-N-(p-bromobenzenesulfonyl)-9-acridinium carboxamide trifluoromethanesulfonate, 10-methyl-N-phenyl-N-(p-nitrobenzenesulfonyl)-9-acridinium carboxamide trifluoromethanesulfonate, 10-methyl-N-phenyl-N-(o-nitrobenzenesulfonyl)-9-acridinium carboxamide trifluoromethanesulfonate, or 10-methyl-N-phenyl-N-trifluoromethanesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate.

14. The chemiluminescent compound as recited in claim 1 wherein said compound is 10-methyl-N-isopropyl-N-tosyl-9-acridinium carboxamide trifluoromethanesulfonate, 10-methyl-N-isopropyl-N-(p-bromobenzenesulfonyl)-9-acridinium carboxamide trifluoromethanesulfonate, 10-methyl-N-isopropyl-N-(o-nitrobenzenesulfonyl)-9-acridinium carboxamide trifluoromethanesulfonate, or 10-methyl-N-isopropyl-N-trifluoromethanesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate.

15. The chemiluminescent compound as recited in claim 1 wherein said compound is 10-methyl-N-butyl-N-(2,4,6 trimethylbenzenesulfonyl)-9-acridinium carboxamide trifluoromethanesulfonate, 10-methyl-N-butyl-N-(2,4,6-tri-isopropyl-benzenesulfonyl)-9-acridinium carboxamide trifluoromethanesulfonate, 10-methyl-N-butyl-N-tosyl-9-acridinium-carboxamide trifluoromethanesulfonate, 10-methyl-N-butyl-N-(p-bromobenzenesulfonyl)-9-acridinium carboxamide trifluoromethanesulfonate, 10-methyl-N-butyl-N-(o-nitrophenylsulfonyl)-9-acridinium carboxamide trifluoromethanesulfonate, 10-methyl-N-butyl-N-(p-nitrobenzenesulfonyl)-9-acridinium carboxamide trifluoromethanesulfonate, 10-methyl-N-butyl-N-(2,4-dinitrobenzenesulfonyl)-9-acridinium carboxamide trifluoromethanesulfonate, or 10-methyl-N-allyl-N-tosyl-9-acridinium carboxamide trifluoromethanesulfonate.

16. The chemiluminescent compound as recited in claim 1 wherein said compound is 6-[N-tosyl-N-(2-carboxyethyl)]-phenanthridinecarboxamide, methyl ester, 5-methyl-6-[N-tosyl-N-(2-carboxyethyl)]-phenanthridiniumcarboxamide, methyl ester, or 5-methyl-6-[N-tosyl-N-(2-carboxyethyl)]-phenanthridinium-carboxamide.

17. A method for preparation of a chemiluminescent compound comprising the steps of: contacting an amine identified by the formula

X^3-R-NH_2

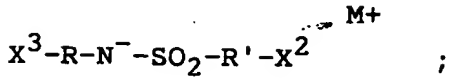
with a sulfonylhalide identified by the formula

W-SO_2-R'-X^2

in an inert solvent in the presence of base to form a sulfonamide identified by the formula

$X^3RNHSO_2R'X^2$; and

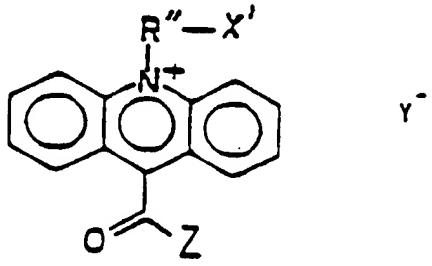
contacting the sulfonamide in an inert solvent in the presence of a base to form a sulfonamide anion identified by the formula



;

and

a) acylating with an activated 9-acridinecarboxylic acid identified by the formula



to produce said chemiluminescent compound identified by the formula

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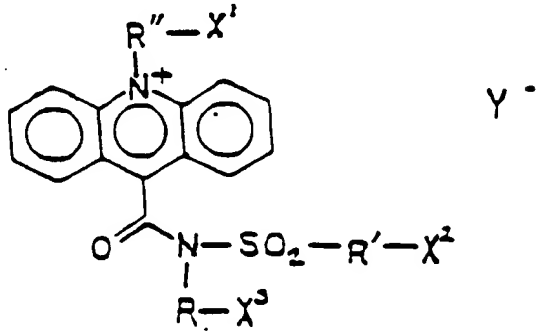
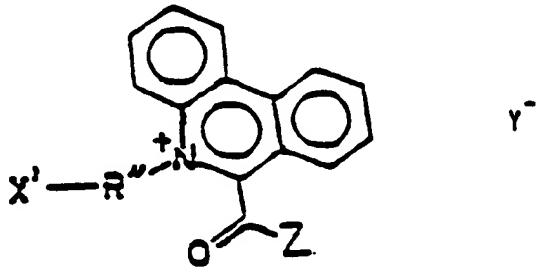


defined in claim 1,

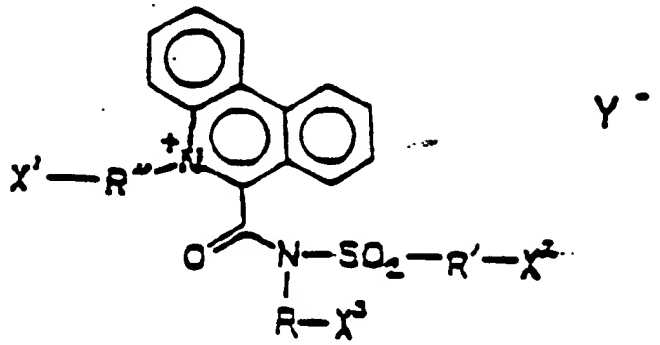
or

10-methyl-N-allyl-N-p-toluenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate; or

b) acylating with an activated phenanthridine-6-carboxylic acid identified by the formula



to produc said chemiluminescent compound identified by the formula



defined in claim 1;

wherein W is selected from the group consisting of chloro and fluoro groups; and

wherein M is selected from the group consisting of Li, Na and K; and

wherein Z is selected from the group consisting of halo, imidazolo, N-hydroxysuccinimidyl and azido groups.

18. A method for preparation of a chemiluminescent compound comprising the steps of:

contacting an amine identified by the formula

X^3-R-NH_2

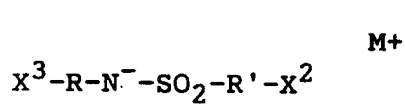
with a sulfonylhalide identified by the formula

W-SO_2-R'-X^2

in an inert solvent in the presence of base to form a sulfonamide identified by the formula

$X^3RNHSO_2R'X^2$; and

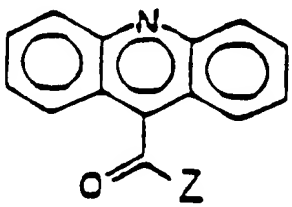
contacting the sulfonamide in an inert solvent in the presence of a base to form a sulfonamide anion identified by the formula



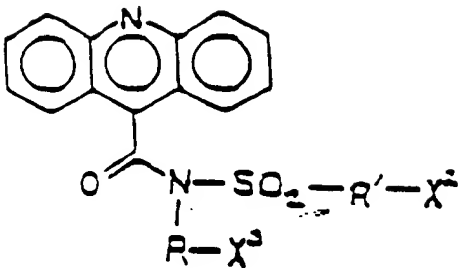
;

and

a) acylating with an activated 9-acridinecarboxylic acid identified by the formula



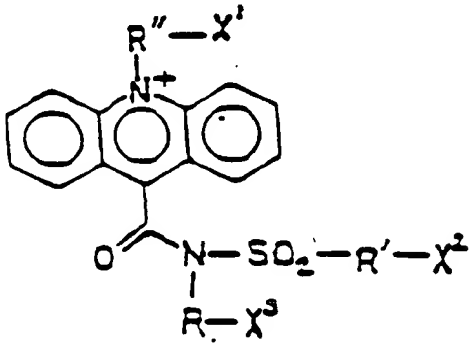
to form a compound identified by the formula



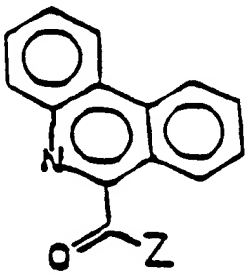
and contacting said compound with an alkylating agent of the formula

Y-R''-X^1

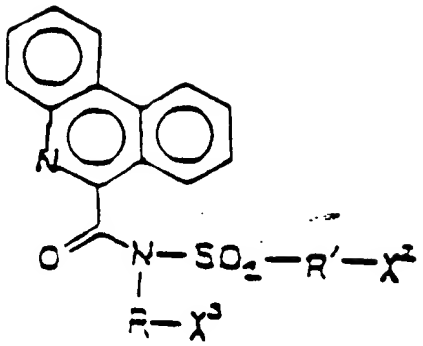
to produce said chemiluminescent compound identified by the formula

I 

defined in claim 1,
or
10-methyl-N-allyl-N-p-toluenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate, or
b) acylating with an activated phenanthridine-6-carboxylic acid identified by the formula



to form a compound identified by the formula

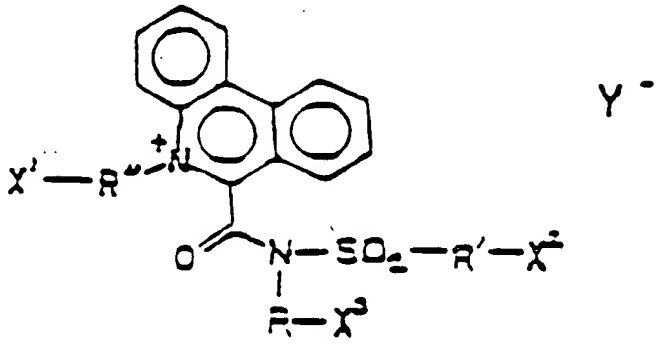


and contacting said compound with an alkylating agent of the formula

Y-R''-X^1

to produce said chemiluminescent compound identified by the formula

II



defined in Claim 1;

wherein W is selected from the group consisting of chloro and fluoro groups; and

wherein M is selected from the group consisting of Li, Na and K; and

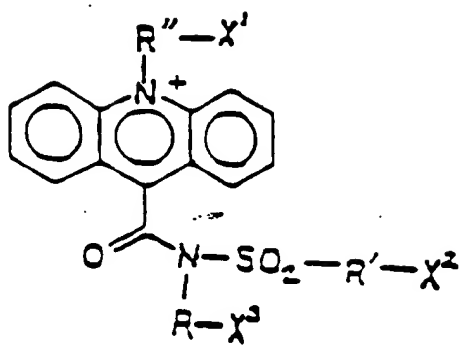
wherein Z is selected from the group consisting of halo, imidazolo, N-hydroxysuccinimidyl and azido groups.

19. The method according to claim 17 or 18 wherein said heteroatom is selected from the group consisting of nitrogen, phosphorus, sulfur and oxygen.

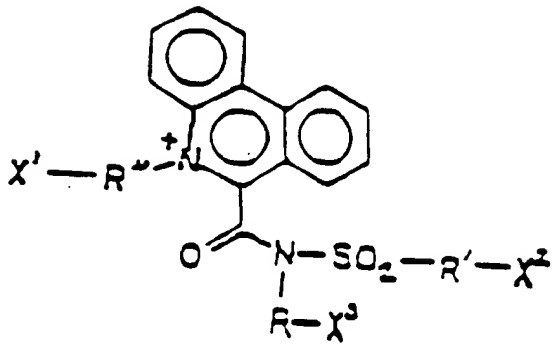
20. A conjugate formed by an antibody or antigen conjugated to a chemiluminescent compound recited in claim 1, with the further proviso that when in said chemiluminescent compound of formula I either one of X^2 and X^3 in R'-X^2 and R-X^3 is carboxy, carboalkoxy, carboxamido or carboaryloxy and the other one of R'-X^2 and R-X^3 is selected from hydrogen, alkyl, aryl or benzyl or such an aryl or benzyl substituted by alkoxy, aryloxy, amino, or hydroxy, then X^1 and R''-X^1 are different from H.

21. A method for performing a chemiluminescent immunoassay to cest for the presence of an antigen or antibody to an antigen as recited in claim 20 comprising the step of exposing a sample to a conjugate as recited in claim 20.

22. A conjugate formed by a nucleic acid probe conjugated to a chemiluminescent compound selected from compounds identified by the formula

I 

and

II 

wherein R, R', and R'' may independently include a member selected from the group consisting of alkylene, arylene, substituted alkylene and substituted arylene groups, such that: one or more hydrogens of said member is replaced by an alkyl, aryl, substituted alkyl, substituted aryl, alkoxy, aryloxy, halo, amino, protected amino, substituted amino, hydroxy, protected hydroxy, oxo, thio, imino, mercapto or substituted mercapto group; or such that one or more carbon atoms of the member is replaced by a heteroatom;

wherein X^1, X^2 and X^3 are independently members of the group consisting of hydrogen, carboxy, carboalkoxyl, carboxamido, carboaryloxy, cyano, carboximido, isocyanato, isothiocyanato, sulfo, sulfonyl halide, carbonyl halide, N-succinimidyloxycarbonyl and N-maleimide groups; or

wherein one of $R'\text{-}X^2$ or $R\text{-}X^3$ can either be a nitro-benzene, provided that the other one is selected from phenyl, iso-propyl, n-butyl or benzyl 5-carboxypentyl, or a dinitro-benzene, provided that the other one is selected from n-butyl and phenyl; and

wherein Y^- is an appropriate counter ion;

with the proviso that $R\text{-}X^3$, $R'\text{-}X^2$ and $R''\text{-}X^1$ may also independently be hydrogen;

and also selected from 10-methyl-N-allyl-N-p-toluenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate.

23. A method for performing a chemiluminescent assay to test for the presence of a nucleic acid as recited in Claim 22 comprising the step of exposing a sample to a conjugate as recited in Claim 22.

Claims for the following Contracting States : AT, ES

1. A method for preparation of a chemiluminescent compound comprising the steps of:
   contacting an amine identified by the formula

   $X^3\text{-}R\text{-}NH_2$

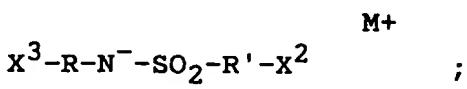
   with a sulfonylhalide identified by th formula

$W\text{-}SO_2\text{-}R'\text{-}X^2$

in an inert solvent in the presence of base to form a sulfonamide identified by the formula

$X^3RNHSO_2R'X^2$; and

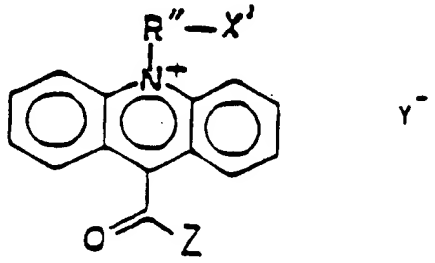
contacting the sulfonamide in an inert solvent in the presence of a base to form a sulfonamide anion identified by the formula



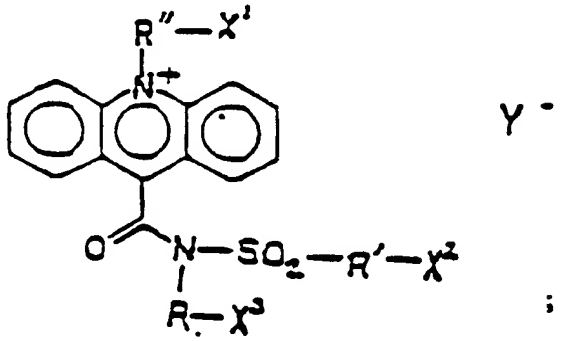
;

and

a) acylating with an activated 9-acridinecarboxylic acid identified by the formula



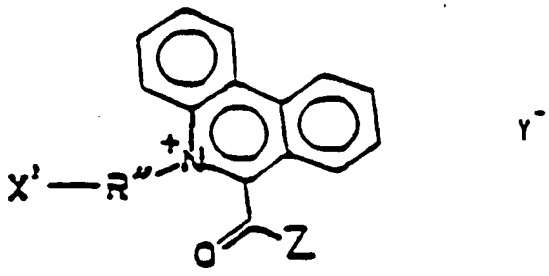
to produce said chemiluminescent compound identified by the formula

I 

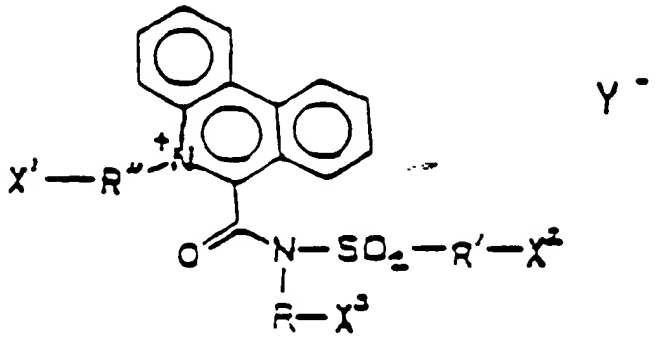
;

or

b) acylating with an activated phenanthridine-6-carboxylic acid identified by the formula

II 

to produce said chemiluminescent compound identified by the formula



wherein R, R' and R" independently comprise a member selected from the group consisting of: alkylene, arylene, substituted alkylene and substituted arylene groups, such that: one or more hydrogens of said member is replaced by an alkyl, aryl, substituted alkyl, substituted aryl, alkoxy, aryloxy, halo, amino, protected amino, substituted amino, hydroxy, protected hydroxy, oxo, thio, imino, mercapto or substituted mercapto group,

or such that one or more carbon atoms of said member is replaced by a heteroatom;

wherein X^1, X^2 and X^3 are independently members of the group consisting of hydrogen, carboxy, carboalkoxyl, carboxamido, carboaryloxy, cyano, carboximido, isocyanato, isothiocyanato, sulfo, sulfonyl halide, carbonyl halide, N-carboxysuccinimide and N-maleimide groups; or

wherein one of $R'-X^2$ or $R-X^3$ can either be a nitro-benzene, provided that the other one is selected from phenyl, iso-propyl, n-butyl or benzyl 5-carboxypentyl, or a dinitro-benzene, provided that the other one is selected from n-butyl and phenyl; and

wherein Y^- is an appropriate counter ion;

wherein W is selected from the group constisting of chloro and fluoro groups; and

wherein M is selected from the group consisting of Li, Na and K; and

wherein Z is selected from the group consisting of halo, imidazolo, N-hydroxysuccinimidyl and azido groups;

provided that $R-X^3$, $R'-X^2$ and $R''-X^1$ may also independently be hydrogen, and

with the further proviso that when in the compounds of formula I in either one of $R'-X^2$ and $R-X^3$, X^2 or X^3 is selected from carbopentachlorophenoxy, carbo-p-nitrophenoxy, carboximido, isothiocyanate, N-maleimide and N-succinimidylcarboxy, and the other one of $R'-X^2$ and $R-X^3$ is selected from hydrogen, alkyl, aryl or benzyl or such aryl or benzyl substituted by alkoxy, aryloxy, amino, or

hydroxy, then X^1 is different from H and $R''-X^1$ is different from H;

and wherein said chemiluminescent compound can also be 10-methyl-N-allyl-N-p-toluenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate.

2. A method for preparation of a chemiluminescent compound comprising the steps of:

contacting an amine identified by the formula

X^3-R-NH_2

with a sulfonylhalide identified by the formula

W-SO_2-R'-X^2

in an inert solvent in the presence of base to form a sulfonamide identified by the formula

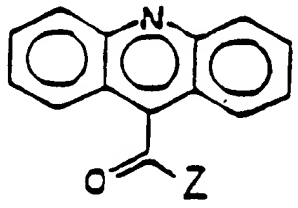
$X^3RNHSO_2R'X^2$; and

contacting the sulfonamide in an inert solvent in the presence of a base to form a sulfonamide anion identified by the formula

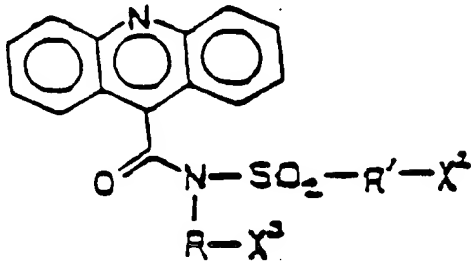
$$X^3\text{-}R\text{-}N^-\text{-}SO_2\text{-}R'\text{-}X^2 \quad M^+ \quad ;$$

and

a) acylating with an activated 9-acridinecarboxylic acid identified by the formula



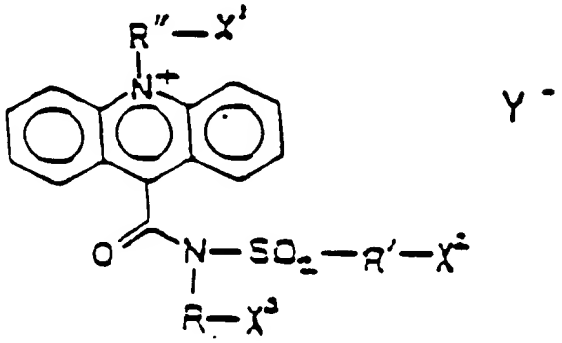
to form a compound identified by the formula



and contacting said compound with an alkylating agent of the formula

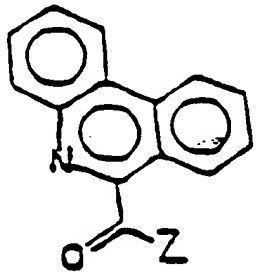
Y-R''-X^1

to produce said chemiluminescent compound identified by the formula

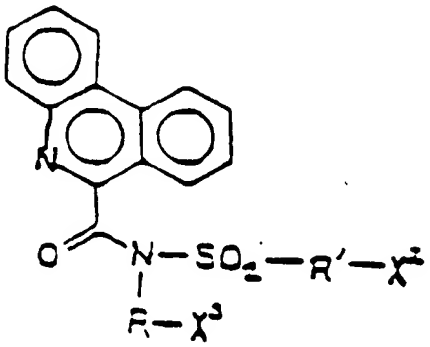
I  ;

or

b) acylating with an activated phenanthridine-6-carboxylic acid identified by the formula



to form a compound identified by the formula

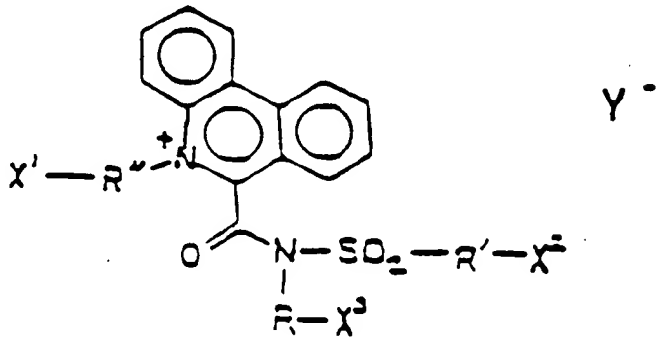


and contacting said compound with an alkylating agent of the formula

Y-R''-X¹

to produce said chemiluminescent compound identified by the formula

II



wherein R, R' and R'' are independently selected from the group consisting of: alkylene, arylene, substituted alkylene and substituted arylene groups, such that: one or more hydrogens of said member is replaced by an alkyl, aryl, substituted alkyl, substituted aryl, alkoxy, aryloxy, halo, amino, protected amino, substituted amino, hydroxy, protected hydroxy, oxo, thio, imino, mercapto or substituted mercapto group,

or such that one or more carbon atoms of said member is replaced by a heteroatom;

wherein X^1, X^2 and X^3 are independently members of the group consisting of hydrogen, carboxy, carboalkoxyl, carboxamido, carboaryloxy, cyano, carboximide, isocyanato, isothiocyanato, sulfo,

sulfonyl halide, carbonyl halide, N-carboxysuccinimide and N-maleimide groups; or

wherein one of R'-X^2 or R-X^3 can either be a nitro-benzene, provided that the other one is selected from phenyl, iso-propyl, n-butyl or benzyl 5-carboxypentyl, or a dinitro-benzene, provided that the other one is selected from n-butyl and phenyl; and

wherein Y^- is an appropriate counter ion;

wherein W is selected from the group consisting of chloro and fluoro groups; and

wherein M is selected from the group consisting of Li, Na and K; and

wherein Z is selected from the group consisting of halo, imidazolo, N-hydroxysuccinimidyl and azido groups;

provided that R-X^3, R'-X^2 and R''-X^1 may also independently be hydrogen, and

with the further proviso that when in the compounds of formula I in either one of R'-X^2 and R-X^3, X^2 or X^3 is selected from carbopentachlorophenoxy, carbo-p-nitrophenoxy, carboximido, isothiocyanate, N-maleimide and N-succinimidylcarboxy, and the other one of R'-X^2 and R-X^3 is selected from hydrogen, alkyl, aryl or benzyl or such aryl or benzyl substituted by alkoxy, aryloxy, amino, or

hydroxy, then X^1 is different from H and R''-X^1 is different from H;

and wherein said chemiluminescent compound can also be 10-methyl-N-allyl-N-p-toluenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate.

3. The method according to claim 1 or 2 wherein said heteroatom is selected from the group consisting of nitrogen, phosphorus, sulfur and oxygen.

4. The method as recited in claim 1 or 2
wherein said compound is 10-methyl-N-[2-carboxy-ethyl]-N-tosyl-9-acridinium carboxamide.

5. The method as recited in claim 1 or 2
wherein said compound is 10-methyl-N-(4-carboxy-butyl)-N-tosyl-9-acridinium carboxamide.

6. The method as recited in claim 1 or 2
wherein said compound is 10-methyl-N-(5-carboxy-pentyl)-N-tosyl-9-acridinium carboxamide.

7. The method as recited in claim 1 or 2
wherein said compound is 10-(3-sulfopropyl)-N-(2-carboxyethyl)-N-tosyl-9-acridinium carboxamide.

8. The method as recited in claim 1 or 2
wherein said compound is 10-(3-sulfopropyl)-N-(3-sulfopropyl)-N-tosyl-9-acridinium carboxamide.

9. The method as recited in claim 1 or 2
wherein said compound is selected from 10-methyl-N-phenyl-N-tosyl-9-acridinium carboxamide trifluoromethanesulfonate, 10-methyl-N-phenyl-N-(p-bromobenzenesulfonyl)-9-acridinium carboxamide trifluoromethanesulfonate, 10-methyl-N-phenyl-N-(p-nitrobenzenesulfonyl)-9-acridinium carboxamide trifluoromethanesulfonate, 10-methyl-N-phenyl-N-(o-nitrobenzenesulfonyl)-9-acridinium carboxamide trifluoromethanesulfonate, or 10-methyl-N-phenyl-N-trifluoromethanesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate.

10. The method as recited in claim 1 or 2
wherein said compound is
10-methyl-N-isopropyl-N-tosyl-9-acridinium carboxamide trifluoromethanesulfonate, 10-methyl-N-isopropyl-N-(p-bromobenzenesulfonyl)-9-acridinium carboxamide trifluoromethanesulfonate, 10-methyl-N-isopropyl-N-(o-nitrobenzenesulfonyl)-9-acridinium carboxamide trifluoromethanesulfonate, or 10-methyl-N-isopropyl-N-trifluoromethanesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate.

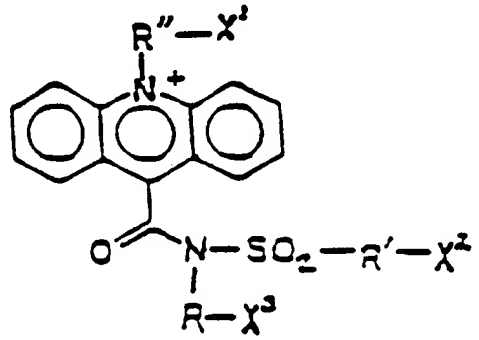
11. The method as recited in claim 1 or 2 wherein said compound is
10-methyl-N-butyl-N-(2,4,6 trimethylbenzenesulfonyl)-9-acridiniumcarboxamide trifluoromethanesulfonate, 10-methyl-N-butyl-N-(2,4,6,-tri-isopropyl-benzenesulfonyl)-9-acridinium carboxamide trifluoromethanesulfonate, 10-methyl-N-butyl-N-tosyl-9-acridinium-carboxamide trifluoromethanesulfonate, 10-methyl-N-butyl-N-(p-bromobenzenesulfonyl)-9-acridinium carboxamide trifluoromethanesulfonate, 10-methyl-N-butyl-N-(o-nitrophenylsulfonyl)-9-acridinium carboxamide trifluoromethanesulfonate,

10-methyl-N-butyl-N-(p-nitrobenzenesulfonyl)-9-acridinium carboxamide trifluoromethanesulfonate, 10-methyl-N-butyl-N-(2,4-dinitrobenzenesulfonyl)-9-acridinium carboxamide trifluoromethanesulfonate, or 10-methyl-N-allyl-N-tosyl-9-acridinium carboxamide trifluoromethanesulfonate.

12. The method as recited in claim 1 or 2 wherein said compound is 6-{N-tosyl-N-(2-carboxyethyl)]-phenanthridinecarboxamide, methyl ester, 5-methyl-6-{N-tosyl-N-(2-carboxyethyl)]-phenanthridiniumcarboxamide, methyl ester or 5-methyl-6-{N-tosyl-N-(2-carboxyethyl)]-phenanthridiniumcarboxamide.

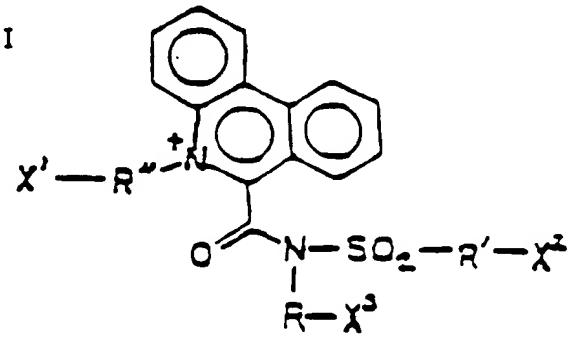
13. A method for performing a chemiluminescent immunoassay to test for the presence of an antigen or antibody to an antigen comprising the step of exposing a sample to a conjugate formed by an antibody or antigen conjugated to a chemiluminescent compound selected from compounds identified by the formula

I



and

II



wherein R, R' and R'' may independently include a member selected from the group consisting of alkylene, arylene, substituted alkylene and substituted arylene groups, such that: one or more hydrogens of said member is replaced by an alkyl, aryl, substituted alkyl, substituted aryl, alkoxy, aryloxy, halo, amino, protected amino, substituted amino, hydroxy, protected hydroxy, oxo, thio, imino, mercapto or substituted mercapto group; or such that one or more carbon atoms of the member is replaced by a heteroatom;

wherein X^1, X^2, and X^3 are independently members of the group consisting of hydrogen, carboxy, carboalkoxyl, carboxamido, carboaryloxy, cyano, carboximido, isocyanato, isothiocyanato, sulfo, sulfonyl halide, carbonyl halide, N-succinimidyloxycarbonyl and N-maleimide groups; or

wherein one of $R'-X^2$ or $R-X^3$ can either be a nitro-benzene, provided that the other one is selected from phenyl, iso-propyl, n-butyl or benzyl 5-carboxypentyl, or a dinitro-benzene, provided that the other one is selected from n-butyl and phenyl; and

wherein Y^- is an appropriate counter ion;

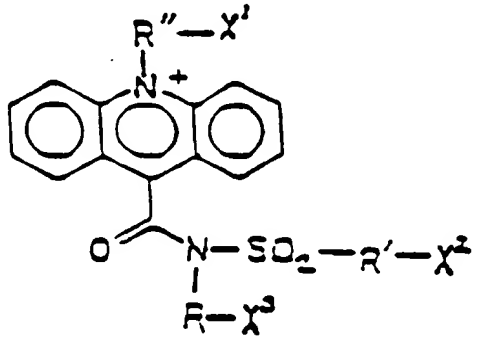
with the proviso that $R-X^3$, $R'-X^2$ and $R''-X^1$ may also independently be hydrogen, and

with the further proviso that when in the compounds of formula I in either one of $R'-X^2$ and $R-X^3$, X^2

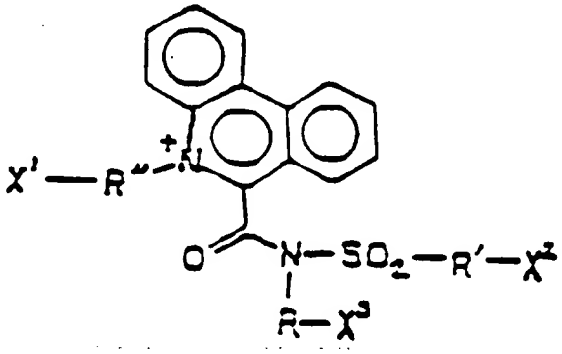
or X^3 is selected from carbopentachlorophenoxy, carbo-p-nitrophenoxy, carboximido, isothiocyanate, N-maleimide and N-succinimidylcarboxy, and the other one of R'-X^2 and R-X^3 is selected from hydrogen, alkyl, aryl, or benzyl, or such aryl or benzyl substituted by alkoxy, aryloxy, amino, or hydroxy, then X^1 is different from H and R''-X^1 is different from H;

and also selected from 10-methyl-N-allyl-N-p-toluenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate.

14. A method for performing a chemiluminescent assay to test for the presence of a nucleic acid comprising the step of exposing a sample to a conjugate formed by a nucleic acid probe conjugated to a chemiluminescent compound selected from compounds identified by the formula

I 

and

II 

wherein R, R' and R'' may independently include a member selected from the group consisting of alkylene, arylene, substituted alkylene and substituted arylene groups, such that: one or more hydrogens of said member is replaced by an alkyl, aryl, substituted alkyl, substituted aryl, alkoxy, aryloxy, halo, amino, protected amino, substituted amino, hydroxy, protected hydroxy, oxo, thio, imino, mercapto or substituted mercapto group; or such that one or more carbon atoms of the member is replaced by a heteroatom;

wherein X^1, X^2 and X^3 are independently members of the group consisting of hydrogen, carboxy, carboalkoxy, carboxamido, carboaryloxy, cyano, carboximido, isocyanato, isothiocyanato, sulfo, sulfonyl halide, carbonyl halide, N-succinimidyloxycarbonyl and N-maleimide groups; or

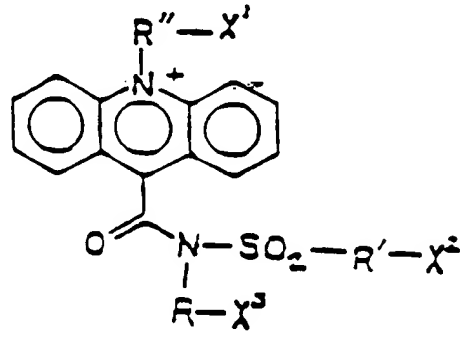
wherein one of R'-X^2 or R-X^3 can either be a nitro-benzene, provided that the other one is selected from phenyl, iso-propyl, n-butyl or benzyl 5-carboxypentyl, or a dinitro-benzene, provided that the other one is selected from n-butyl and phenyl; and

wherein Y^- is an appropriate counter ion;

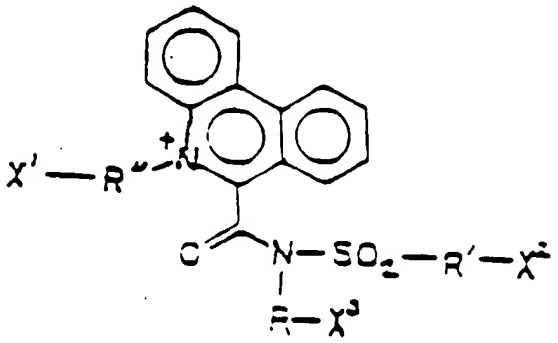
with the proviso that R-X^3, R'-X^2 and R''-X^1 may also independently be hydrogen;

and also selected from 10-methyl-N-allyl-N-p-toluenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate.

15. A method for preparation of a conjugate of an antibody or antigen and a chemiluminescent compound comprising the steps of covalently coupling an antibody or antigen to a chemiluminescent compound selected from compounds identified by the formulae

I 

and

II 

wherein R, R' and R" independently comprise a member selected from the group consisting of alkylene, arylene, substituted alkylene, and substituted arylene groups such that:

one or more hydrogens of said member is replaced by an alkyl, aryl, substituted alkyl, substituted aryl, alkoxy, aryloxy, halo, amino, protected amino, substituted amino, hydroxy, protected hydroxy, oxo, thio, imino, mercapto or substituted mercapto group,

or such that one or more carbon atoms of said member is replaced by a heteroatom;

wherein X^1, X^2 and X^3 are independently members of the group consisting of hydrogen, carboxy, carboalkoxyl, carboxamido, carboaryloxy, cyano, carboximido, isocyanato, isothiocyanato, sulfo, sulfonyl halide, carbonyl halide, N-succinimidylcarboxy and N-maleimide groups; or

wherein one of $R'-X^2$ or $R-X^3$ can either be a nitro-benzene, provided that the other one is selected from phenyl, iso-propyl, n-butyl or benzyl 5-carboxypentyl, or a dinitro-benzene, provided that the other one is selected from n-butyl and phenyl; and

wherein Y^- is an appropriate counter ion;

with the proviso that $R-X^3$, $R'-X^2$ and $R''-X^1$ may also independently be hydrogen, and

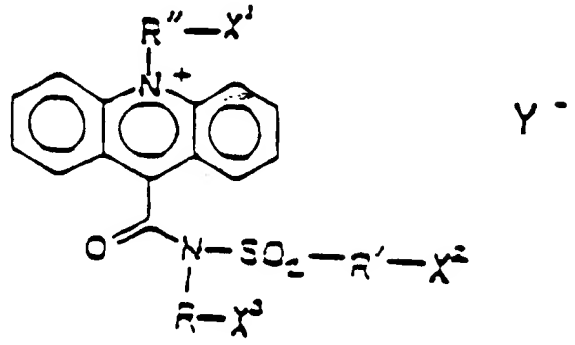
with the further proviso that when in the compounds of formula I in either one of $R'-X^2$ and $R-X^3$, X^2 or X^3 is selected from carbopentachlorophenoxy, carbo-p-nitrophenoxy, carboximido, isothiocyanate, N-maleimide, N-succinimidylcarboxy, carboxy, carboalkoxy, carboxamido and carboaryloxy, and the other one of $R'-X^2$ and

$R-X^3$ is selected from hydrogen, alkyl, aryl or benzyl, or such aryl or benzyl substituted by alkoxy, aryloxy, amino, or hydroxy,

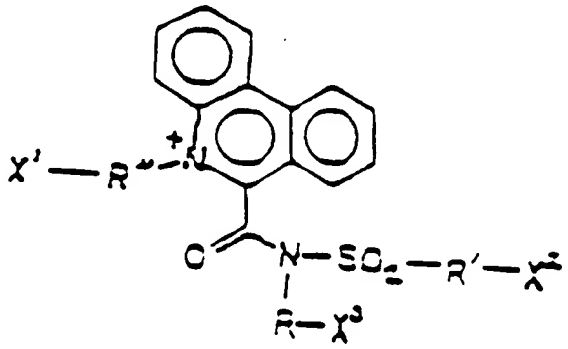
then X^1 is different from H and $R''-X^1$ is different from H;

and also selected from 10-methyl-N-allyl-N-p-toluenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate.

16. A method for preparation of a conjugate of a nucleic acid probe and a chemiluminescent compound comprising the steps of covalently coupling a nucleic acid probe to a chemiluminescent compound selected from compounds identified by the formula

I 

and

II 

wherein R, R', and R'' may independently include a member selected from the group consisting of alkylene, arylene, substituted alkylene and substituted arylene groups, such that: one or more hydrogens of said member is replaced by an alkyl, aryl, substituted alkyl, substituted aryl, alkoxy, aryloxy, halo, amino, protected amino, substituted amino, hydroxy, protected hydroxy, oxo, thio, imino, mercapto or substituted mercapto group; or such that one or more carbon atoms of the member is replaced by a heteroatom;

wherein X^1, X^2 and X^3 are independently members of the group consisting of hydrogen, carboxy, carboalkoxyl, carboxamido, carboaryloxy, cyano, carboximido, isocyanato, isothiocyanato, sulfo, sulfonyl halide, carbonyl halide, N-succinimidyloxycarbonyl and N-maleimide groups; or

wherein one of $R'\text{-}X^2$ or $R\text{-}X^3$ can either be a nitro-benzene, provided that the other one is selected from phenyl, iso-propyl, n-butyl or benzyl 5-carboxypentyl, or a dinitro-benzene, provided that the other one is selected from n-butyl and phenyl; and

wherein Y^- is an appropriate counter ion;

with the proviso that $R\text{-}X^3$, $R'\text{-}X^2$ and $R''\text{-}X^1$ may also independently be hydrogen;

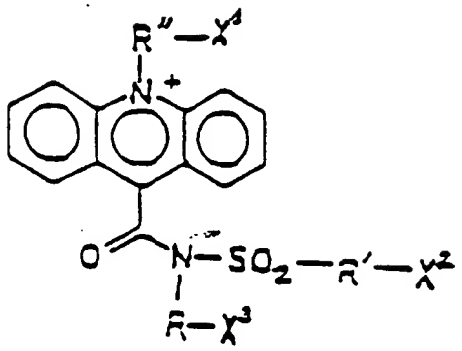
and also selected from 10-methyl-N-allyl-N-p-toluenesulfonyl-9-acridinium carboxamide trifluoromethanesulfonate.

Patentansprüche

Patentansprüche für folgende Vertragsstaaten : BE, CH, DE, FR, GB, GR, IT, LI, LU, NL, SE

1. Eine chemilumineszente Verbindung, die aus Verbindungen entsprechend den folgenden Formeln gewählt ist

I



und

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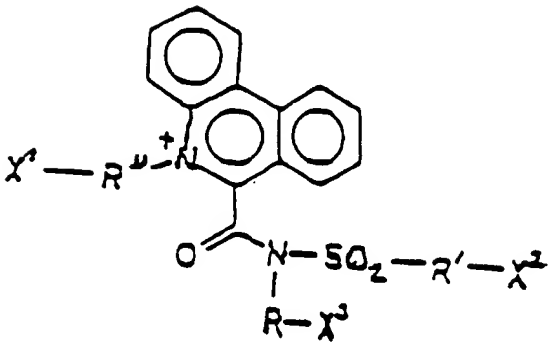


wobei R, R' und R'' unabhängig voneinander ein Glied aus der Gruppe bestehend aus Alkylen-, Arylen-, substituierten Alkylen- und substituierten Arylengruppen enthalten derart, daß:

einer oder mehrere Wasserstoffe des Glieds durch eine Alkyl-, Aryl-, substituierte Alkyl-, substituierte Aryl-, Alkoxy-, Aryloxy-, Halogen-, Amino-, geschützte Amino-, substituierte Amino-, Hydroxy-, geschützte Hydroxy-, Oxo-, Thio-, Imino-, Mercapto- oder substituierte Mercaptogruppe ersetzt sind;

oder derart, daß ein oder mehrere Kohlenstoffatome des Glieds durch ein Heteroatom ersetzt ist;

wobei X^1, X^2 und X^3 unabhängig voneinander Glieder der Gruppe bestehend aus Wasserstoff, Carboxy-, Carboalkoxyl-, Carboxamido-, Carboaryloxy-, Cyano-, Carboximido-, Isocyanat-, Isothiocyanat-, Sulfo-, Sulfonylhalogenid-, Carbonylhalogenid-, N-Succinimidylcarboxy- und der N-Maleinimidgruppe sind; oder

wobei einer der Reste R'-X^2 oder R-X^3 entweder ein Nitrobenzol ist, vorausgesetzt, daß der andere aus Phenyl, Iso-Propyl, n-B oder Benzyl-5-carboxypentyl gewählt ist, oder aber einer ist ein Dinitrobenzol, vorausgesetzt, daß der andere Rest aus n-Butyl oder Phenyl gewählt ist; und

wobei Y^- ein geeignetes Gegenion ist;

vorausgesetzt, daß R-X^3, R'-X^2 und R''-X^1 ebenfalls unabhängig voneinander Wasserstoff sein können, und

unter dem weiteren Vorbehalt, daß, wenn in den Verbindungen nach Formel I entweder in R'-X^2 oder R-X^3, X^2 oder X^3 aus einer Carbopentachlorphenoxy-, Carbo-p-nitrophenoxy-, Carboximido, Isothiocyanat-, N-Maleinimid- und N-Succinimidylcarboxygruppe gewählt ist, und der andere Rest aus R'-X^2 und R-X^3 aus Wasserstoff, Alkyl, Aryl oder Benzyl, oder solchem Aryl oder Benzyl, das durch Alkoxy, Aryloxy, Amino oder Hydroxy substituiert ist, gewählt ist;

dann X^1 kein Wasserstoff und R''-X^1 kein Wasserstoff ist;

und außerdem aus 10-Methyl-N-allyl-N-p-toluolsulfonyl-9-acridiniumcarboxamidtrifluormethansulfonat.

2. Die chemilumineszente Verbindung nach Anspruch 1, wobei Y^- ein Gegenion, gewählt aus der Gruppe bestehend aus Sulfat, Alkylsulfat, Halogensulfat, Halogenborat, Halogenacetat, Halogenphosphat, Phosphat, Halogenid und Trifluormethansulfonat ist.

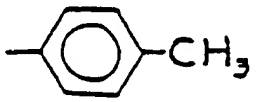
3. Die chemilumineszente Verbindung nach Anspruch 1, wobei das Heteroatom aus der Grupp bestehend aus Stickstoff, Phosphor, Schwefel und Sauerstoff gewählt ist.

4. Die chemilumineszente Verbindung nach Anspruch 1, wobei R, R' und R'' unabhängig voneinander die Formel

$-(CH_2)_n-$

haben, mit n = 0 - 50.

5. Die chemilumineszente Verbindung nach Anspruch 1, wobei R'' gleich $-CH_2-$ ist, X^1 gleich -H ist, und R'-X^2 durch die folgende Formel beschrieben wird:

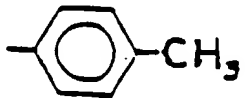


6. Die chemilumineszente Verbindung nach Anspruch 5, wobei die Verbindung 10-Methyl-N-[2-carboxyethyl]-N-tosyl-9-acridiniumcarboxamid ist.

7. Die chemilumineszente Verbindung nach Anspruch 5, wobei die Verbindung 10-Methyl-N-[4-carboxybutyl]-N-tosyl-9-acridiniumcarboxamid ist.

8. Die chemilumineszente Verbindung nach Anspruch 5, wobei die Verbindung 10-Methyl-N-[5-carboxypentyl]-N-tosyl-9-acridiniumcarboxamid ist.

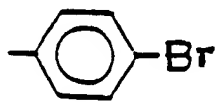
9. Die chemilumineszente Verbindung nach Anspruch 1, wobei R'' gleich $-(CH_2)_3-$ ist, X^1 gleich $-SO_3-$ ist, und R'-X^2 durch die folgende Formel beschrieben wird:



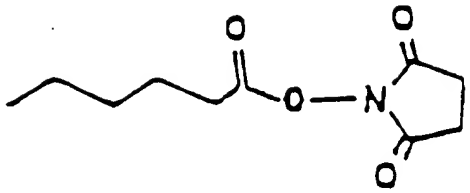
10. Die chemilumineszente Verbindung nach Anspruch 9, wobei die Verbindung 10-(3-Sulfopropyl)-N-(2-carboxyethyl)-N-tosyl-9-acridiniumcarboxamid ist.

11. Die chemilumineszente Verbindung nach Anspruch 9, wobei die Verbindung 10-(3-Sulfopropyl)-N-(3-sulfopropyl)-N-tosyl-9-acridiniumcarboxamid ist.

12. Die chemilumineszente Verbindung nach Anspruch 1, wobei R'-X^2 durch die folgende Formel beschrieben wird:



und wobei R-X^3 durch di folgende Formel beschrieben wird



13. Die chemilumineszente Verbindung nach Anspruch 1, wobei die Verbindung aus 10-Methyl-N-phenyl-N-tosyl-9-acridiniumcarboxamidtrifluormethansulfonat, 10-Methyl-N-phenyl-N-(p-brombenzolsulfonyl)-9-acridiniumcarboxamidtrifluormethansulfonat, 10-Methyl-N-phenyl-N-(p-nitrobenzolsulfonyl)-9-acridiniumcarboxamidtrifluormethansulfonat, 10-Methyl-N-phenyl-N-(o-nitrobenzolsulfonyl)-9-acridiniumcarboxamidtrifluormethansulfonat oder 10-Methyl-N-phenyl-N-trifluormethansulfonyl-9-acridiniumcarboxamidtrifluormethansulfonat gewählt ist.

14. Die chemilumineszente Verbindung nach Anspruch 1, wobei die Verbindung 10-Methyl-N-isopropyl-N-tosyl-9-acridiniumcarboxamidtrifluormethansulfonat, 10-Methyl-N-isopropyl-N-(p-brombenzolsulfonyl)-9-acridiniumcarboxamidtrifluormethansulfonat, 10-Methyl-N-isopropyl-N-(o-nitrobenzolsulfonyl)-9-acridiniumcarboxamidtrifluormethansulfonat oder 10-Methyl-N-isopropyl-N-trifluormethansulfonyl-9-acridiniumcarboxamidtrifluormethansulfonat ist.

15. Die chemilumineszente Verbindung nach Anspruch 1, wobei die Verbindung 10-Methyl-N-butyl-N-(2,4,6-trimethylbenzolsulfonyl)-9-acridiniumcarboxamidtrifluormethansulfonat, 10-Methyl-N-butyl-N-(2,4,6-triisopropylbenzolsulfonyl)-9-acridiniumcarboxamidtrifluormethansulfonat, 10-Methyl-N-butyl-N-tosyl-9-acridiniumcarboxamidtrifluormethansulfonat,10-Methyl-N-butyl-N-(p-brombenzolsulfonyl)-9-acridiniumcarboxamidtrifluormethansulfonat, 10-Methyl-N-butyl-N(o-nitrophenylsulfonyl)-9-acridiniumcarboxamidtrifluormethansulfonat,10-Methyl-N-butyl-N(p-nitrobenzolsulfonyl)-9-acridiniumcarboxamidtrifluormethansulfonat, 10-Methyl-N-butyl-(2,4-dinitrobenzolsulfonyl)-9-acridiniumcarboxamidtrifluormethansulfonat oder 10-Methyl-N-allyl-N-tosyl-9-acridiniumcarboxamidtrifluormethansulfonat ist.

16. Die chemilumineszente Verbindung nach Anspruch 1, wobei die Verbindung 6-[N-Tosyl-N-(2-carboxyethyl)]-phenanthridincarboxamid, Methylester, 5-Methyl-6-[-N-tosyl-N-(2-carboxyethyl)]-phenanthridiniumcarboxamid, Methylester oder 5-Methyl-6-[N-tosyl-N-(2-carboxyethyl)]-phenanthridiniumcarboxamid ist.

17. Verfahren zur Darstellung einer chemilumineszenten Verbindung, das folgende Schritten umfaßt:
Zusammenbringen eines Amins mit der Formel

X^3-R-NH_2

mit einem Sulfonylhalogenid mit der Formel

W-SO_2-R'-X^2

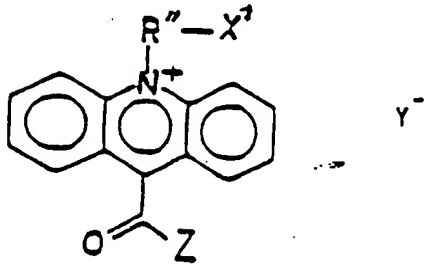
in einem inerten Lösungsmittel in Gegenwart einer Base unter Bildung eines Sulfonamids mit der Formel

$X^3RNHSO_2R'X^2$; und

Eintragen des Sulfonamids in ein inertes Lösungsmittel in Gegenwart einer Base unter Bildung eines Sulfonamidanions mit der Formel

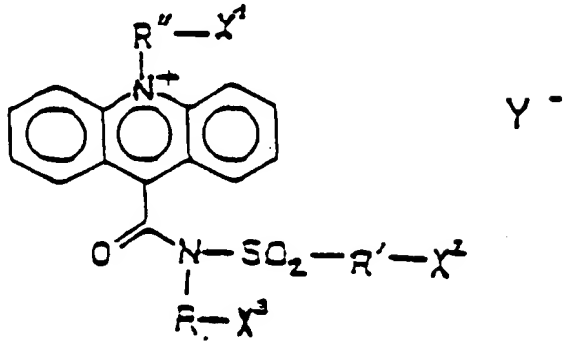
X^3-R-N^--SO_2-R'-$X^{2\ M+}$; und

a) Acylierung mit einer aktivierten 9-Acridincarbonsäure mit der Formel

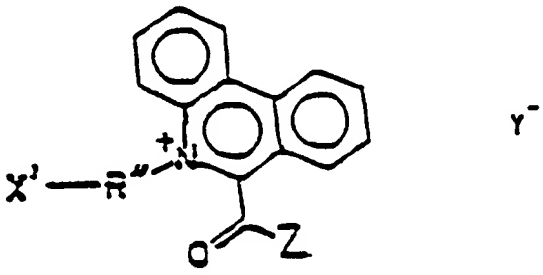


zur Darstellung der chemilumineszenten Verbindung mit der Formel

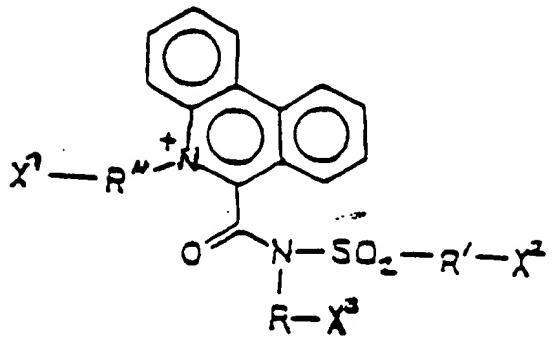
I



wie in Anspruch 1 definiert,
oder von
10-Methyl-N-allyl-N-p-toluolsulfonyl-9-acridiniumcarboxamidtrifluormethansulfonat; oder
b) Acylierung mit einer aktivierten Phenanthridin-6-carbonsäure mit der Formel



zur Darstellung der chemilumineszenten Verbindung mit der Formel

II  Y^-

wie in Anspruch 1 definiert;
wobei W aus der Gruppe bestehend aus Chlor- und Fluorgruppen gewählt ist; und
wobei M aus der Gruppe bestehend aus Li, Na und K gewählt ist; und
wobei Z aus der Gruppe bestehend aus Halogen-, Imidazol-, N-Hydroxysuccinimidyl- und Azidgruppen gewählt ist.

18. Verfahren zur Darstellung einer chemilumineszenten Verbindung, bestehend aus den Schritten:
Zusammenbringen eines Amins mit der Formel

X^3-R-NH_2

mit einem Sulfonylhalogenid mit der Formel

W-SO_2-R'-X^2

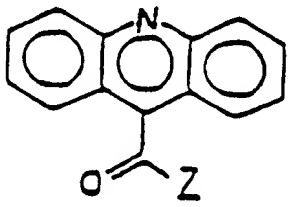
in einem inerten Lösungsmittel in Gegenwart einer Base unter Bildung eines Sulfonamids mit der Formel

$X^3RNHSO_2R'X^2$; und

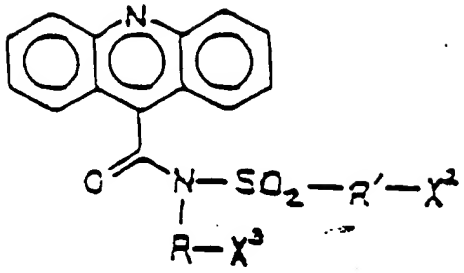
Eintragen des Sulfonamids in ein inertes Lösungsmittel in Gegenwart einer Base unter Bildung eines Sulfonamidanions mit der Formel

X^3-R-N^--SO_2-R'-X^2 $^{M+}$; und

a) Acylierung mit einer aktivierten 9-Acridincarbonsäure mit der Formel



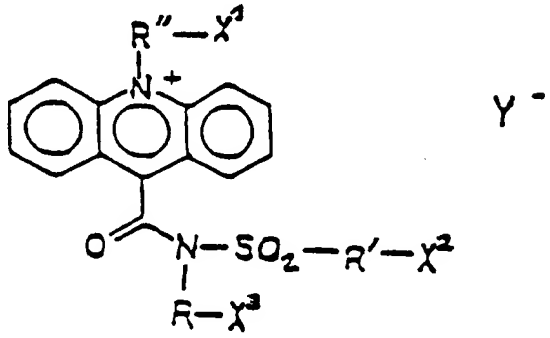
zur Darstellung einer Verbindung mit der Formel



und Zusammenbringen dieser Verbindung mit einem alkylierenden Agens mit der Formel

Y-R''-X^1

zur Darstellung einer chemilumineszenten Verbindung mit der Formel



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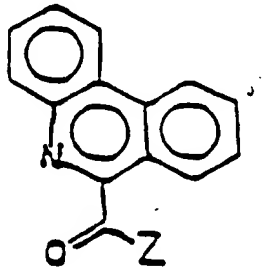
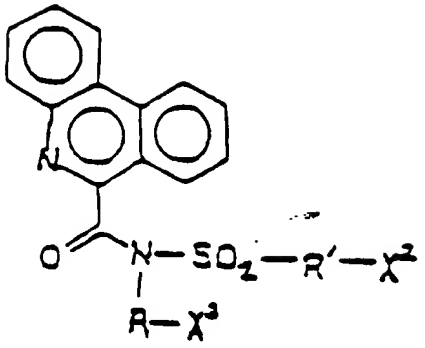
wie in Anspruch 1 definiert,
oder von
10-Methyl-N-allyl-N-p-toluolsulfonyl-9-acridiniumcarboxamidtrifluormethansulfonat, oder

b) Acylierung mit einer aktivierten Phenanthridin-6-carbonsäure mit der Formel



zur Darstellung einer Verbindung mit der Formel

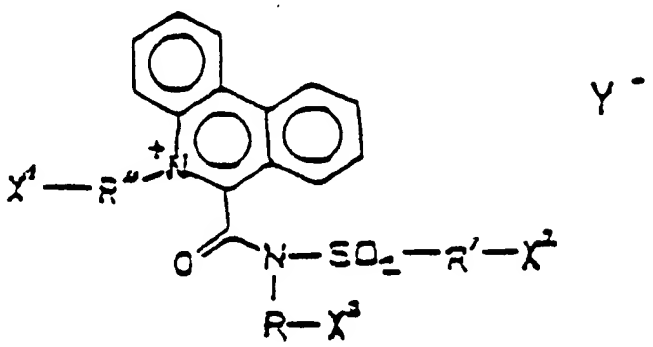


und Zusammenbringen dieser Verbindung mit einem alkylierenden Agens mit der Formel

Y-R''-X^1

zur Darstellung der chemilumineszenten Verbindung mit der Formel

II



wie in Anspruch 1 definiert;

wobei W aus der Gruppe bestehend aus Chlor- und Fluorgruppen gewählt ist; und

wobei M aus der Gruppe bestehend aus Li, Na und K gewählt ist; und

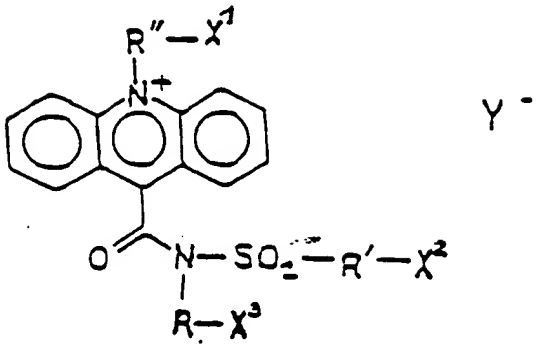
wobei Z aus der Gruppe bestehend aus Halogen-, Imidazol-, N-Hydroxysuccinimidyl- und Azidgruppen gewählt ist.

19. Verfahren nach Anspruch 17 oder 18, wobei das Heteroatom aus der Gruppe bestehend aus Stickstoff, Phosphor, Schwefel und Sauerstoff gewählt ist.

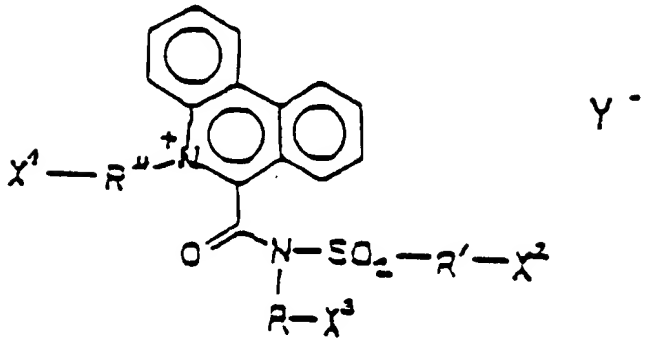
20. Ein Konjugat, das von einem an eine chemilumineszentee Verbindung entsprechend Anspruch 1 konjugiertem Antikörper oder Antigen gebildet wird, unter der weiteren Voraussetzung, daß, wenn in der chemilumineszenten Verbindung nach Formel I entweder X^2 oder X^3 in R'-X^2 oder R'-X^3 eine Carboxy-, Carboalkoxy-, Carboxamid- oder Carboaryloxygruppe ist, und wenn der jeweils andere Rest aus R'-X^2 und R-X^3 aus Wasserstoff, Alkyl, Aryl oder Benzyl oder einem solchen Aryl oder Benzyl gewählt ist, das durch eine Alkoxy-, Aryloxy-, Amino- oder Hydroxygruppe substituiert ist, X^1 und R''-X^1 nicht Wasserstoff sind.

21. Verfahren zur Durchführung eines Chemilumineszenz-Immunoassays zum Nachweis auf Anwesenheit eines Antigens oder Antikörpers gegen ein Antigen nach Anspruch 20, umfassend den Schritt des Aussetzens einer Probe gegenüber einem Konjugat nach Anspruch 20.

22. Ein Konjugat, das aus einer an eine chemilumineszente Verbindung konjugierte Nucleinsäuresonde gebildet ist, wobei die chemilumineszente Verbindung aus Verbindungen entsprechend den folgenden Formeln gewählt ist:

I 

und

II 

wobei R, R' und R'' unabhängig voneinander ein Glied, gewählt aus der Gruppe bestehend aus Alkylen-, Arylen-, substituierten Alkylen- und substituierten Arylengruppen enthalten, derart daß ein oder mehrere Wasserstoffe dieses Glieds durch eine Alkyl-, Aryl-, substituierte Alkyl-, substituierte Aryl-, Alkoxy-, Aryloxy-, Halogen-, Amino-, geschützte Amino-, substituierte Amino-, Hydroxy-, geschützte Hydroxy-, Oxo-, Thio-, Imino-, Mercapto- oder substituierte Mercaptogruppen ersetzt sind, oder daß ein oder mehrere Kohlenstoffatome des Glieds durch ein Heteroatom ersetzt sind;
wobei X^1, X^2 und X^3 unabhängig voneinander Glieder der Gruppe bestehend aus Wasserstoff, Carboxy-, Carboalkoxyl-, Carboxamido-, Carboaryloxy-, Cyano-, Carboximido-, Isocyanat-, Isothiocyanat-, Sulfo-, Sulfonylhalogenid-, Carbonylhalogenid-, N-Succinimidyloxycarbonyl- und der N-Maleinimidgruppe sind; oder

wobei einer der Reste R'-X^2 oder R-X^3 entweder ein Nitrobenzol ist, vorausgesetzt, daß der andere Rest aus Phenyl, Iso-Propyl, n-Butyl oder Benzyl-5-carboxypentyl gewählt ist, oder aber einer ist ein Dinitrobenzol ist, vorausgesetzt, daß der andere Rest aus n-Butyl oder Phenyl gewählt ist; und

wobei Y^- ein geeignetes Gegenion ist;

vorausgesetzt, daß R-X^3, R'-X^2 und R''-X^1 außerdem unabhängig voneinander Wasserstoff sein können;

und außerdem aus 10-Methyl-N-allyl-N-p-toluolsulfonyl-9-acridiniumcarboxamidtrifluormethansulfonat.

23. Verfahren zur Durchführung eines Chemilumineszenzassays zum Nachweis des Vorhandenseins einer Nucleinsäure nach Anspruch 22, welches den Schritt des Aussetzens einer Probe gegenüber einem Konjugat nach Anspruch 22 umfaßt.

Patentansprüche für folgende Vertragsstaaten : AT, ES

1. Verfahren zur Darstellung einer chemilumineszenten Verbindung, bestehend aus den Schritten:
Zusammenbringen eines Amins mit der Formel

X^3-R-NH_2

mit einem Sulfonylhalogenid mit der Formel

$W\text{-}SO_2\text{-}R'\text{-}X^2$

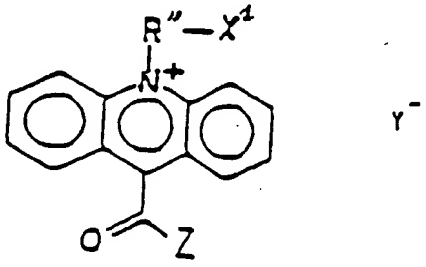
in einem inerten Lösungsmittel in Gegenwart einer Base unter Bildung eines Sulfonamids mit der Formel

$X^3RNHSO_2R'X^2$; und

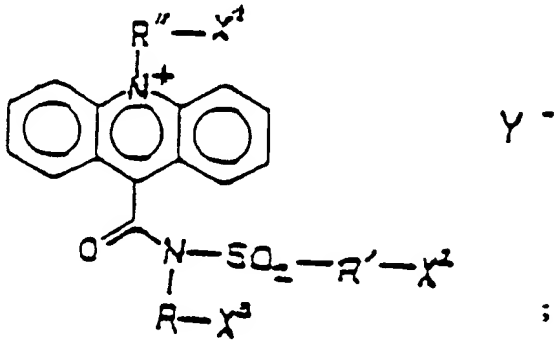
Eintragen des Sulfonamids in ein inertes Lösungsmittel in Gegenwart einer Base unter Bildung eines Sulfonamidanions mit der Formel

$X^3\text{-}R\text{-}N^-\text{-}SO_2\text{-}R'\text{-}X^{2\ M+}$; und

a) Acylierung mit einer aktivierten 9-Acridincarbonsäure mit der Formel

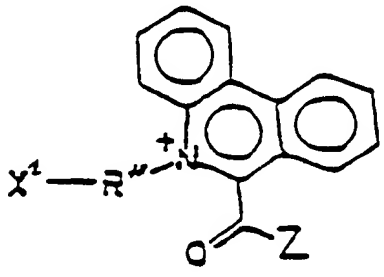


zur Darstellung der chemilumineszenten Verbindung mit der Formel

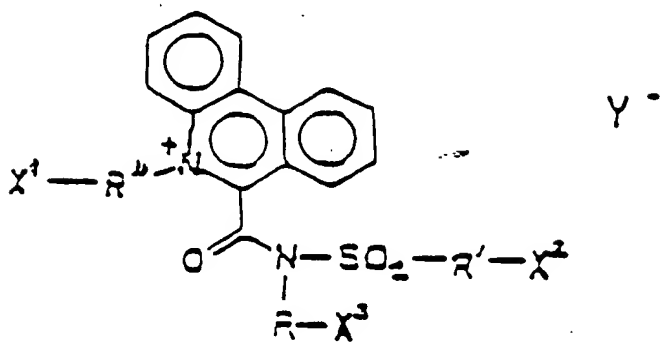
I  ;

oder

b) Acylierung mit einer aktivierten Phenanthridin-6-carbonsäure mit der Formel

II 

zur Darstellung der chemilumineszenten Verbindung mit der Formel



wobei R, R' und R'' unabhängig voneinander ein Glied aus der Gruppe, bestehend aus Alkylen-, Arylen-, substituierten Alkylen- und substituierten Arylengruppen enthalten, derart daß ein oder mehrere Wasserstoffe des Glieds durch eine Alkyl-, Aryl-, substituierte Alkyl-, substituierte Aryl-, Alkoxy-, Aryloxy-, Halogen-, Amino-, geschützte Amino-, substituierte Amino-, Hydroxy-, geschützte Hydroxy-, Oxo-, Thio-, Imino-, Mercapto- oder substituierte Mercaptogruppe ersetzt sind,

oder derart, daß ein oder mehrere Kohlenstoffatome des Glieds durch ein Heteroatom ersetzt sind;

wobei X^1, X^2 und X^3 unabhängig voneinander Glieder der Gruppe bestehend aus Wasserstoff, Carboxy-, Carboalkoxyl-, Carboxamido-, Carboaryloxy-, Cyano-, Carboximido-, Isocyanat-, Isothiocyanat-, Sulfo-, Sulfonylhalogenid-, Carbonylhalogenid-, N-Carboxysuccinimid- und der N-Maleinimidgruppe sind; oder

wobei einer der Reste $R'-X^2$ oder $R-X^3$ entweder ein Nitrobenzol ist, vorausgesetzt, daß der andere Rest aus Phenyl, Iso-Propyl, n-Butyl oder Benzyl-5-carboxypentyl ge ist, oder aber einer ein Dinitrobenzol ist, vorausgesetzt, daß der andere aus n-Butyl oder Phenyl gewählt ist; und

wobei Y^- ein geeignetes Gegenion ist;

wobei W aus der Gruppe bestehend aus Chlor- und Fluorgruppen gewählt ist; und

wobei M aus der Gruppe bestehend aus Li, Na und K gewählt ist; und

wobei Z aus der Gruppe bestehend aus Halogen-, Imidazol-, N-Hydroxysuccinimidyl- und Azidgruppen gewählt ist;

vorausgesetzt, daß $R-X^3$, $R'-X^2$ und $R''-X^1$ ebenfalls unabhängig voneinander Wasserstoff sein können, und

unter dem weiteren Vorbehalt, daß, wenn in den Verbindungen nach Formel I entweder in $R'-X^2$ oder $R-X^3$, X^2 oder X^3 aus einer Carbopentachlorphenoxy-, Carbo-p-nitrophenoxy-, Carboximido, Isothiocyanat-, N-Maleinimid- und N-Succinimidylcarboxygruppe gewählt ist, und der andere von $R'-X^2$ und $R-X^3$ aus Wasserstoff, Alkyl, Aryl oder Benzyl, oder solchem Aryl oder Benzyl, das durch Alkoxy, Aryloxy, Amino oder Hydroxy substituiert ist, gewählt ist,

dann X^1 kein Wasserstoff und $R''-X^1$ kein Wasserstoff ist;

und wobei die chemilumineszente Verbindung desweiteren 10-Methyl-N-allyl-N-p-toluolsulfonyl-9-acridiniumcarboxamidtrifluormethansulfonat sein kann.

2. Verfahren zur Darstellung einer chemilumineszenten Verbindung, das aus den Schritten besteht:
Zusammenbringen eines Amins mit der Formel

X^3-R-NH_2

mit einem Sulfonylhalogenid mit der Formel

$W-SO_2-R'-X^2$

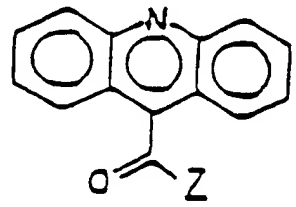
in einem inerten Lösungsmittel in Gegenwart einer Base unter Bildung eines Sulfonamids mit der Formel

$X^3RNHSO_2R'X^2$; und

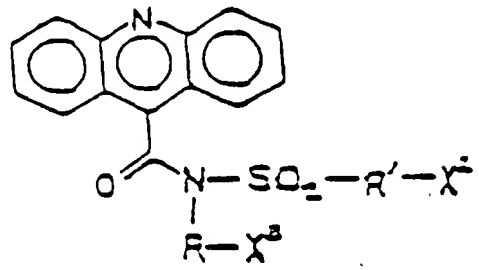
Eintragen des Sulfonamids in ein inertes Lösungsmittel in Gegenwart einer Base unter Bildung eines Sulfonamidanions mit der Formel

$X^3\text{-}R\text{-}N^-\text{-}SO_2\text{-}R'\text{-}X^2\ ^{M+}$; und

a) Acylierung mit einer aktivierten 9-Acridincarbonsäure mit der Formel



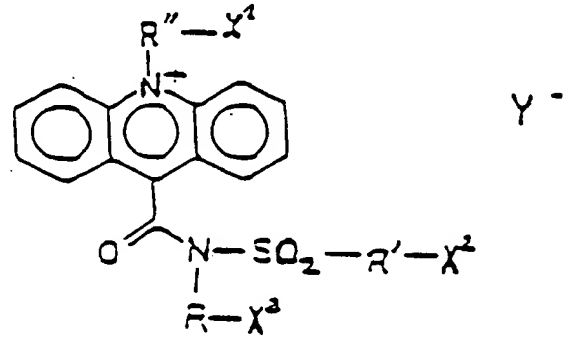
zur Darstellung einer Verbindung mit der Formel



und Zusammenbringen dieser Verbindung mit einem alkylierenden Agens mit der Formel

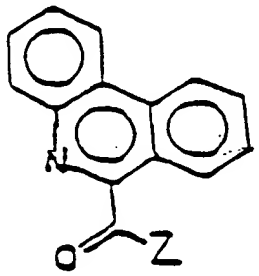
$Y\text{-}R''\text{-}X^1$

zur Darstellung der chemilumineszenten Verbindung mit der Formel

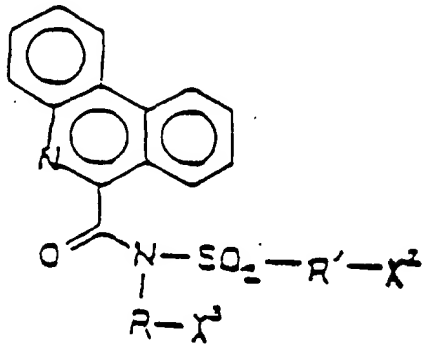
I  ;

oder

b) Acylierung mit einer aktivierten Phenanthridin-6-carbonsäure mit der Formel



zur Darstellung einer Verbindung mit der Formel

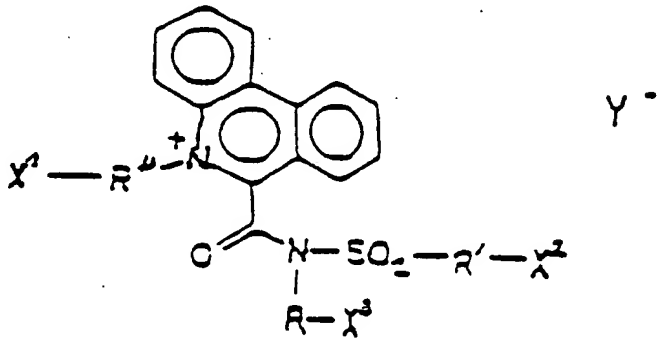


und Zusammenbringen dieser Verbindung mit einem alkylierenden Agens mit der Formel

$Y\text{-}R''\text{-}X^1$

zur Darstellung der chemilumineszenten Verbindung mit der Formel

II



wobei R, R' und R'' unabhängig voneinander ein Glied enthalten aus der Gruppe bestehend aus Alkylen-, Arylen-, substituierten Alkylen- und substituierten Arylengruppen, derart daß ein oder mehrere Wasserstoffe des Glieds durch eine Alkyl-, Aryl-, substituierte Alkyl-, substituiert Aryl-, Alkoxy-, Aryloxy-, Halogen-, Amino-, geschützte Amino-, substituierte Amino-, Hydroxyl-, geschützte Hydroxyl-, Oxo-, Thio-, Imino-, Mercapto- oder substituierte Mercaptogruppe ersetzt sind,

oder derart, daß ein oder mehrere Kohlenstoffatome des Glieds durch ein Heteroatom ersetzt sind;

wobei X^1, X^2 und X^3 unabhängig voneinander Glieder der Gruppe bestehend aus Wasserstoff,

Carboxy-, Carboalkoxy-, Carboxamido-, Carboaryloxy-, Cyano-, Carboximido-, Isocyanat-, Isothiocyanat-, Sulfo-, Sulfonylhalogenid-, Carbonylhalogenid-, N-Carboxysuccinimid- und der N-Maleinimidgruppe sind; oder

wobei einer der Reste R'-X^2 oder R-X^3 entweder ein Nitrobenzol ist, vorausgesetzt, daß der andere aus Phenyl, Iso-Propyl, n-Butyl oder Benzyl-5-carboxypentyl gewählt ist, oder aber einer ist ein Dinitrobenzol, vorausgesetzt, daß der andere aus n-Butyl oder Phenyl gewählt ist; und

wobei Y^- ein geeignetes Gegenion ist;

wobei W aus der Gruppe bestehend aus Chlor- und Fluorgruppen gewählt ist; und

wobei M aus der Gruppe bestehend aus Li, Na und K gewählt ist; und

wobei Z aus der Gruppe bestehend aus Halogen-, Imidazol-, N-Hydroxysuccinimidyl- und Azidgruppen gewählt ist;

vorausgesetzt, daß R-X^3, R'-X^2 und R''-X^1 ebenfalls unabhängig voneinander Wasserstoff sein können, und

unter dem weiteren Vorbehalt, daß, wenn in den Verbindungen nach Formel I entweder in R'-X^2 oder R-X^3, X^2 oder X^3 aus einer Carbopentachlorphenoxy-, Carbo-p-nitrophenoxy-, Carboximido, Isothiocyanat-, N-Maleinimid- und N-Succinimidylcarboxygruppe gewählt ist, und der andere Rest aus R'-X^2 und R-X^3 aus Wasserstoff, Alkyl, Aryl oder Benzyl, oder solchem Aryl oder Benzyl, das durch Alkoxy, Aryloxy, Amino oder Hydroxyl substituiert ist, gewählt ist,

dann X^1 kein Wasserstoff und R''-X^1 kein Wasserstoff ist;

und wobei die chemilumineszente Verbindung auch 10-Methyl-N-allyl-N-p-toluolsulfonyl-9-acridiniumcarboxamidtrifluormethansulfonat sein kann.

3. Verfahren nach Anspruch 1 oder 2, wobei das Heteroatom aus der Gruppe bestehend aus Stickstoff, Phosphor, Schwefel oder Sauerstoff gewählt ist.

4. Verfahren nach Anspruch 1 oder 2, wobei die Verbindung 10-Methyl-N-[2-carboxyethyl]-N-tosyl-9-acridiniumcarboxamid ist.

5. Verfahren nach Anspruch 1 oder 2, wobei die Verbindung 10-Methyl-N-[4-carboxybutyl]-N-tosyl-9-acridiniumcarboxamid ist.

6. Verfahren nach Anspruch 1 oder 2, wobei die Verbindung 10-Methyl-N-[5-carboxypentyl]-N-tosyl-9-acridiniumcarboxamid ist.

7. Verfahren nach Anspruch 1 oder 2, wobei die Verbindung 10-(3-Sulfopropyl)-N-(2-carboxyethyl)-N-tosyl-9-acridiniumcarboxamid ist.

8. Verfahren nach Anspruch 1 oder 2, wobei die Verbindung 10-(3-Sulfopropyl)-N-(3-sulfopropyl)-N-tosyl-9-acridiniumcarboxamidist.

9. Verfahren nach Anspruch 1 oder 2, wobei die Verbindung aus 10-Methyl-N-phenyl-N-tosyl-9-acridiniumcarboxamidtrifluormethansulfonat, 10-Methyl-N-phenyl-N-(p-brombenzolsulfonyl)-9-acridiniumcarboxamidtrifluormethansulfonat, 10-Methyl-N-phenyl-N-(p-nitrobenzolsulfonyl)-9-acridiniumcarboxamidtrifluormethansulfonat, 10-Methyl-N-phenyl-N-(o-nitrobenzolsulfonyl)-9-acridiniumcarboxamidtrifluormethansulfonat oder 10-Methyl-N-phenyl-N-trifluormethansulfonyl-9-acridiniumcarboxamidtrifluormethansulfonat gewählt ist.

10. Verfahren nach Anspruch 1 oder 2, wobei die Verbindung 10-Methyl-N-isopropyl-N-tosyl-9-acridiniumcarboxamidtrifluormethansulfonat, 10-Methyl-N-isopropyl-N-(p-brombenzolsulfonyl)-9-acridiniumcarboxamidtrifluormethansulfonat,10-Methyl-N-isopropyl-N-(o-nitrobenzolsulfonyl)-9-acridiniumcarboxamidtrifluormethansulfonat oder 10-Methyl-N-isopropyl-N-trifluormethansulfonyl-9-acridiniumcarboxamidtrifluormethansulfonat ist.

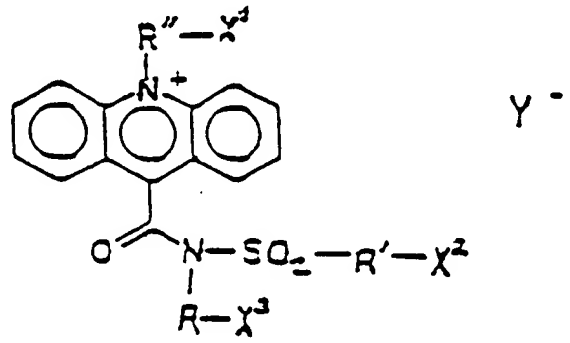
11. Verfahren nach Anspruch 1 oder 2, wobei die Verbindung 10-Methyl-N-butyl-N-(2,4,6-Trimethylbenzolsulfonyl)-9-acridiniumcarboxamidtrifluormethansulfonat, 10-Methyl-N-butyl-N-(2,4,6-triisopropylbenzolsulfonyl)-9-acridiniumcarboxamidtrifluormethansulfonat, 10-Methyl-N-butyl-N-tosyl-9-acridiniumcarboxamidtrifluormethansulfonat,10-Methyl-N-butyl-N-(p-brombenzolsulfonyl)-9-acridiniumcarboxamidtrifluormethansulfonat, 10-Methyl-N-butyl-N-(o-nitrophenylsulfonyl)-9-acridinium-

carboxamidtrifluormethansulfonat,10-Methyl-N-butyl-N-(p-nitrobenzolsulfonyl)-9-acridiniumcarboxamidtrifluormethansulfonat, 10-Methyl-N-butyl-N-(2,4-dinitrobenzolsulfonyl)-9-acridiniumcarboxamidtrifluormethansulfonat oder 10-Methyl-N-allyl-N-tosyl-9-acridiniumcarboxamidtrifluormethansulfonat ist.

12. Verfahren nach Anspruch 1 oder 2, wobei die Verbindung 6-[N-Tosyl-N-(2-carboxyethyl)]-phenanthridincarboxamid, Methyl-Ester, 5-Methyl-6-[N-tosyl-N-(2-carboxyethyl)]-phenanthridiniumcarboxamid, Methyl-Ester oder 5-Methyl-6-[N-tosyl-N-(2-carboxyethyl)]-phenanthridiniumcarboxamid ist.

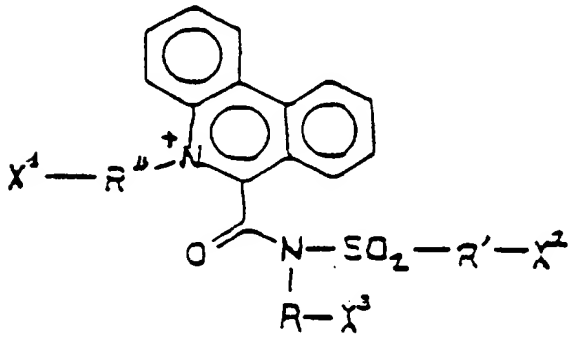
13. Verfahren zur Durchführung eines Chemilumineszenz-Immunoassays zum Nachweis auf Anwesenheit eines Antigens oder eines Antikörpers gegen ein Antigen, das den Schritt beinhaltet, eine Probe einem Konjugat, das aus einem Antikörper oder Antigen, welches an eine chemilumineszente Verbindung konjugiert ist, gebildet wird, auszusetzen, wobei die chemilumineszente Verbindung aus Verbindungen entsprechend den folgenden Formeln gewählt ist:

I



und

II



wobei R, R' und R'' unabhängig voneinander ein Glied, gewählt aus der Gruppe bestehend aus Alkylen-, Arylen-, substituierten Alkylen- und substituierten Arylengruppen enthalten, derart daß ein oder mehrere Wasserstoffe dieses Glieds durch eine Alkyl-, Aryl, substituierte Alkyl-, substituierte Aryl-, Alkoxy-, Aryloxy-, Halogen-, Amino-, geschützte Amino-, substituierte Amino-, Hydroxy-, geschützte Hydroxy-, Oxo-, Thio-, Imino-, Mercapto- oder substituierte Mercaptogruppen ersetzt sind, oder daß ein oder mehrere Kohlenstoffatome des Glieds durch ein Heteroatom ersetzt sind;

wobei X^1, X^2 und X^3 unabhängig voneinander Glieder der Gruppe bestehend aus Wasserstoff, Carboxy-, Carboalkoxyl-, Carboxamido-, Carboaryloxy-, Cyano-, Carboximido-, Isocyanat-, Isothiocyanat-, Sulfo-, Sulfonylhalogenid-, Carbonylhalogenid-, N-Succinimidyloxycarbonyl- und der N-Maleinimidgruppe sind; oder

wobei einer der Reste R'-X^2 oder R-X^3 entweder ein Nitrobenzol ist, vorausgesetzt, daß der andere aus Phenyl, Iso-Propyl, n-Butyl oder Benzyl-5-carboxypentyl gewählt ist, oder aber einer ein Dinitrobenzol ist, vorausgesetzt, daß der andere aus n-Butyl oder Phenyl gewählt ist; und

wobei Y^- ein geeignetes Gegenion ist;

vorausgesetzt, daß R-X^3, R'-X^2 und R''-X^1 außerdem unabhängig voneinander Wasserstoff sein

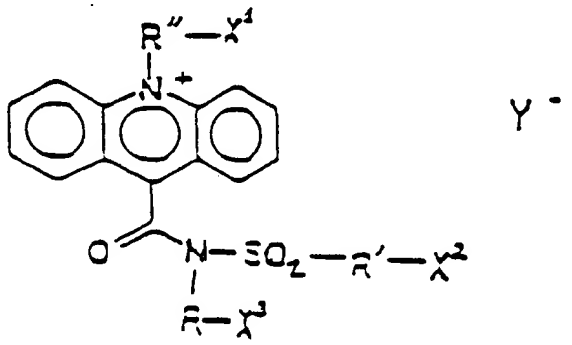
können, und

unter dem weiteren Vorbehalt, daß, wenn in den Verbindungen nach Formel I entweder in R'-X^2 oder R-X^3, X^2 oder X^3 aus einer Carbopentachlorphenoxy-, Carbo-p-nitrophenoxy-, Carboximido, Isothiocyanat-, N-Maleinimid- und N-Succinimidylcarboxygruppe gewählt ist, und der andere Rest aus R'-X^2 und R-X^3 aus Wasserstoff, Alkyl, Aryl oder Benzyl, oder solchem Aryl oder Benzyl, das durch Alkoxy, Aryloxy, Amino oder Hydroxy substituiert ist, gewählt ist,

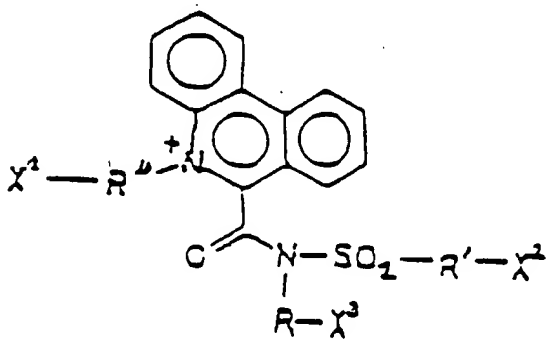
dann X^1 kein Wasserstoff und R''-X^1 kein Wasserstoff ist,

und auch aus 10-Methyl-N-allyl-N-p-toluolsulfonyl-9-acridiniumcarboxamidtrifluormethansulfonat.

14. Verfahren für die Durchführung eines Chemilumineszenzassays zum Nachweis auf Anwesenheit einer Nucleinsäure, das den Schritt umfaßt, eine Probe einem Konjugat auszusetzen, daß aus einer Nucleinsäuresonde gebildet ist, die an eine chemilumineszente Verbindung konjugiert ist, welche aus Verbindungen entsprechend den folgenden Formeln gewählt ist:

I  Y^-

und

II  Y^-

wobei R, R' und R'' unabhängig voneinander ein Glied, gewählt aus der Gruppe bestehend aus Alkylen-, Arylen-, substituierten Alkylen- und substituierten Arylengruppen enthalten, derart daß ein oder mehrere Wasserstoffe dieses Glieds durch eine Alkyl-, Aryl-, substituierte Alkyl-, substituierte Aryl-, Alkoxy-, Aryloxy-, Halogen-, Amino-, geschützte Amino-, substituierte Amino-, Hydroxy-, geschützte Hydroxy-, Oxo-, Thio-, Imino-, Mercapto- oder substituierte Mercaptogruppen ersetzt sind, oder daß ein oder mehrere Kohlenstoffatome des Glieds durch ein Heteroatom ersetzt sind;

wobei X^1, X^2 und X^3 unabhängig voneinander Glieder der Gruppe bestehend aus Wasserstoff, Carboxy-, Carboalkoxy-, Carboxamido-, Carboaryloxy-, Cyano-, Carboximido-, Isocyanat-, Isothiocyanat-, Sulfo-, Sulfonylhalogenid-, Carbonylhalogenid-, N-Succinimidyloxycarbonyl- und der N-Maleinimidgruppe sind; oder

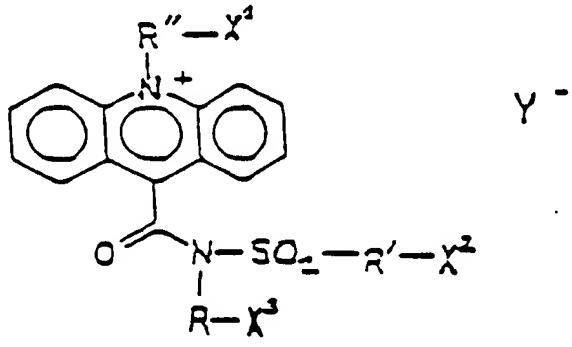
wobei einer der Reste R'-X^2 oder R-X^3 entweder ein Nitrobenzol ist, vorausgesetzt, daß der andere aus Phenyl, Iso-Propyl, n-Butyl oder Benzyl-5-carboxypentyl gewählt ist, oder aber einer ist ein Dinitrobenzol, vorausgesetzt, daß der andere aus n-Butyl oder Phenyl gewählt ist; und

wobei Y^- ein geeign tes Gegenion ist

vorausgesetzt, daß R-X^3, R'-X^2 und R''-X^1 außerdem unabhängig voneinander Wasserstoff sein können,
und auch aus 10-Methyl-N-allyl-N-p-toluolsulfonyl-9-acridiniumcarboxamidtrifluormethansulfonat.

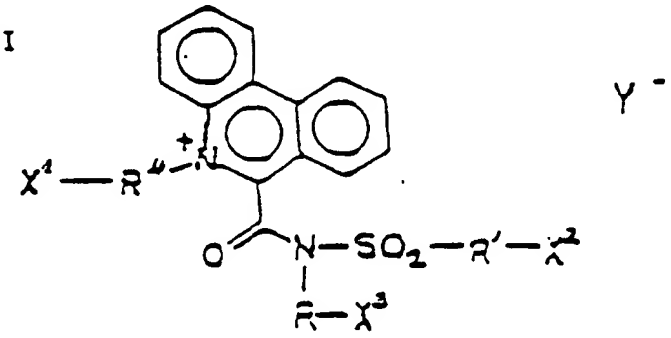
15. Verfahren für die Darstellung eines Konjugats aus einem Antikörper oder Antigen und einer chemilumineszenten Verbindung, bestehend aus den Schritten kovalente Kupplung eines Antikörpers oder Antigens an eine chemilumineszente Verbindung, die aus Verbindungen nach einer der Formeln gewählt ist:

I



und

II

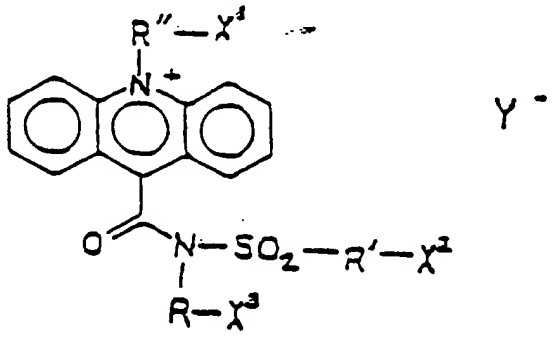


wobei R, R' und R'' unabhängig voneinander ein Glied, gewählt aus der Gruppe bestehend aus Alkylen-, Arylen-, substituierten Alkylen- und substituierten Arylengruppen enthalten derart, daß:
ein oder mehrere Wasserstoffe dieses Glieds durch eine Alkyl-, Aryl-, substituierte Alkyl-, substituierte Aryl-, Alkoxy-, Aryloxy-, Halogen-, Amino-, geschützte Amino-, substituierte Amino-, Hydroxy-, geschützte Hydroxy-, Oxo-, Thio-, Imino-, Mercapto- oder substituierte Mercaptogruppen ersetzt sind,
oder, daß ein oder mehrere Kohlenstoffatome des Glieds durch ein Heteroatom ersetzt sind;
wobei X^1, X^2 und X^3 unabhängig voneinander Glieder der Gruppe bestehend aus Wasserstoff, Carboxy-, Carboalkoxy-, Carboxamido-, Carboaryloxy-, Cyano-, Carboximido-, Isocyanat-, Isothiocyanat-, Sulfo-, Sulfonylhalogenid-, Carbonylhalogenid-, N-Succinimidylcarboxy- und N-Maleinimidgruppen sind; oder
wobei einer der Reste R'-X^2 oder R-X^3 entweder ein Nitrobenzol ist, vorausgesetzt, daß der andere aus Phenyl, Iso-Propyl, n-Butyl oder Benzyl-5-carboxypentyl gewählt ist, oder aber einer ein Dinitrobenzol ist, vorausgesetzt, daß der andere aus n-Butyl oder Phenyl gewählt ist; und
wobei Y^- ein geeignetes Gegenion ist;
vorausgesetzt, daß R-X^3, R'-X^2 und R''-X^1 außerdem unabhängig voneinander Wasserstoff sein können, und
unter dem weiteren Vorbehalt, daß, wenn in den Verbindungen nach Formel I entweder in R'-X^2 oder R-X^3, X^2 oder X^3 aus einer Carbopentachlorphenoxy-, Carbo-p-nitrophenoxy-, Carboximido-, Isothiocyanat-, N-Maleinimid-, N-Succinimidylcarboxy-, Carboxy-, Carboalkoxy-, Carboxamido- und Carboaryloxygruppe gewählt ist, und der andere Rest aus R'-X^2 und R-X^3 aus Wasserstoff, Alkyl, Aryl oder Benzyl, oder solchem Aryl oder Benzyl, das durch Alkoxy, Aryloxy, Amino oder Wasserstoff substituiert ist, gewählt ist, dann X^1 kein Wasserstoff und R''-X^1 kein Wasserstoff ist,
und außerdem aus 10-Methyl-N-allyl-N-p-toluolsulfonyl-9-acridiniumcarboxamidtrifluormethansulfo-

nat.

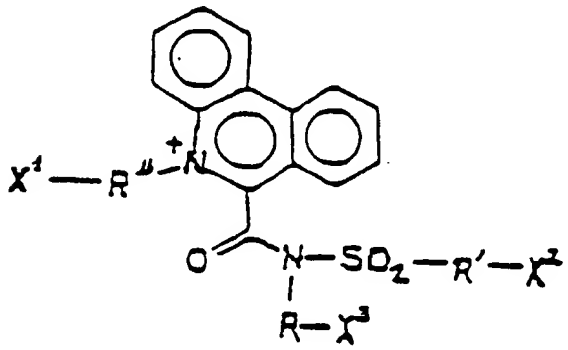
16. Verfahren für die Darstellung eines Konjugats aus einer Nucleinsäuresonde und einer chemilumineszenten Verbindung, bestehend aus den Schritten kovalente Kupplung einer Nucleinsäuresonde an eine chemilumineszente Verbindung, die aus Verbindungen nach einer der Formeln gewählt ist:

I



und

II



wobei R, R' und R'' unabhängig voneinander ein Glied gewählt aus der Gruppe bestehend aus Alkylen-, Arylen-, substituierten Alkylen- und substituierten Arylengruppen enthalten, derart daß ein oder mehrere Wasserstoffe dieses Glieds durch eine Alkyl-, Aryl, substituierte Alkyl-, substituierte Aryl-, Alkoxy-, Aryloxy-, Halogen-, Amino-, geschützte Amino-, substituierte Amino-, Hydroxy-, geschützte Hydroxy-, Oxo-, Thio-, Imino-, Mercapto- oder substituierte Mercaptogruppen ersetzt sind, oder daß ein oder mehrere Kohlenstoffatome des Glieds durch ein Heteroatom ersetzt sind,

wobei X^1, X^2 und X^3 unabhängig voneinander Glieder der Gruppe bestehend aus Wasserstoff, Carboxy-, Carboalkoxyl-, Carboxamido-, Carboaryloxy-, Cyano-, Carboximido-, Isocyanat-, Isothiocyanat-, Sulfo-, Sulfonylhalogenid-, Carbonylhalogenid-, N-Succinimidyloxycarbonyl- und N-Maleinimidgruppen sind; oder

wobei einer der Reste R'-X^2 oder R-X^3 entweder ein Nitrobenzol ist, vorausgesetzt, daß der andere aus Phenyl, Iso-Propyl, n-Butyl oder Benzyl-5-carboxypentyl gewählt ist, oder aber einer ist ein Dinitrobenzol, vorausgesetzt, daß das andere aus n-Butyl oder Phenyl gewählt ist, und

wobei Y^- ein geeignetes Gegenion ist;

vorausg setzt, daß R-X^3, R'-X^2 und R''-X^1 außerdem unabhängig voneinander Wasserstoff sein können,

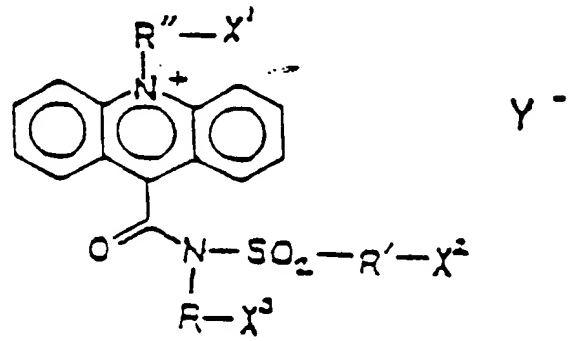
und auch 10-Methyl-N-allyl-N-p-toluolsulfonyl-9-acridiniumcarboxamidtrifluormethansulfonat.

Revendications

Revendications pour les Etats contractants suivants : BE, CH, DE, FR, GB, GR, IT, LI, LU, NL, SE

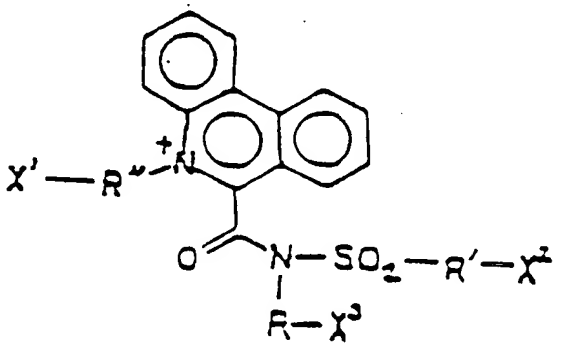
1. Composé chimiluminescent choisi parmi les composés représentés par les formules :

I



et

II



dans lesquelles R, R' et R'' représentent indépendamment un membre choisi dans le groupe constitué des groupes alkylène, arylène, alkylène substitué et arylène substitué, de sorte que :

un ou plusieurs atomes d'hydrogène dudit membre sont remplacés par un groupe alkyle, aryle, alkyle substitué, aryle substitué, alcoxy, aryloxy, halo, amino, amino protégé, amino substitué, hydroxy, hydroxy protégé, oxo, thio, imino, mercapto ou mercapto substitué,

ou qu'un ou plusieurs atomes de carbone dudit membre sont remplacés par un hétéroatome ;

dans lesquelles X^1, X^2 et X^3 représentent indépendamment des membres du groupe constitué de l'hydrogène, des groupes carboxy, carboalcoxyle, carboxamido, carboaryloxy, cyano, carboximido, isocyanato, isothiocyanato, sulfo, halogénure de sulfonyle, halogénure de carbonyle, N-succinimidylcarboxy et N-maléimide ; ou

dans lesquelles l'un des groupes R'-X^2 ou R-X^3 peut être un nitro-benzène, pourvu que l'autre soit choisi parmi les phényle, iso-propyle, n-butyle ou benzyl-5-carboxypentyle ou dinitro-benzène, pourvu que l'autre soit choisi parmi le n-butyle et phényle ; et

dans lesquelles Y^- est un ion approprié de signe opposé ;

à la condition que R-X^3, R'-X^2 et R''-X^1 puissent être aussi indépendamment l'hydrogène, et

à la condition supplémentaire que, lorsque dans les composés de formule I dans l'un ou l'autre des groupes R'-X^2 et R-X^3, on choisit X^2 ou X^3 parmi les carbopentachlorophénoxy, carbo-p-nitrophénoxy, carboximido, isothiocyanate, N-maléimide et N-succinimidylcarboxy, et on choisit l'autre groupe R'-X^2 et R-X^3 parmi les hydrogène, alkyle, aryle ou benzyle, ou de tels aryle ou benzyle sont substitués par un alcoxy, aryloxy, amino ou hydroxy,

alors X^1 est différent de H et R''-X^1 est différent de H ;

et peut être aussi le 10-méthyl-N-allyl-N-p-toluènesulfonyl-9-acridinium-carboxamide trifluorométhanesulfonate.

2. Composé chimilumin scent selon la revendication 1, dans lequel Y^- est un ion de site opposé choisi dans le groupe constitué des sulfate, alkylsulfate, halosulfate, haloborate, haloacétate, halophosphate, phosphate, halogénure et trifluorométhanesulfonate.

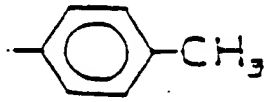
3. Composé chimiluminescent selon la revendication 1, dans lequel on choisit ledit hétéroatome dans le groupe constitué de l'azote, du phosphore, du soufre et de l'oxygène.

4. Composé chimiluminescent selon la revendication 1, dans lequel R, R' et R'' représentent indépendamment la formule :

$-(CH_2)_n-$

dans laquelle n = 0-50.

5. Composé chimiluminescent selon la revendication 1, dans lequel R'' est $-CH_2-$, X^1 est -H, et $R'-X^2$ est représenté par la formule :

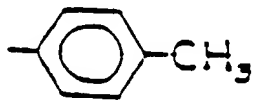


6. Composé chimiluminescent selon la revendication 5, dans lequel ledit composé est le 10-méthyl-N-[2-carboxyéthyl]-N-tosyl-9-acridinium carboxamide.

7. Composé chimiluminescent selon la revendication 5, dans lequel ledit composé est le 10-méthyl-N-(4-carboxybutyl)-N-tosyl-9-acridinium carboxamide.

8. Composé chimiluminescent selon la revendication 5, dans lequel ledit composé est le 10-méthyl-N-(5-carboxypentyl)-N-tosyl-9-acridinium carboxamide.

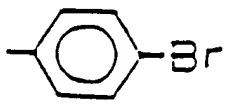
9. Composé chimiluminescent selon la revendication 1, dans lequel R'' est $-(CH_2)_3-$, X' est $-SO_3-$ et $R'-X^2$ est représenté par la formule :



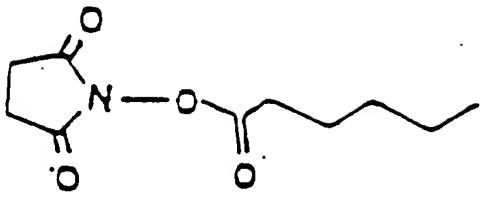
10. Composé chimiluminescent selon la revendication 9, dans lequel ledit composé est le 10-(3-sulfopropyl)-N-(2-carboxyéthyl)-N-tosyl-9-acridinium carboxamide.

11. Composé chimiluminescent selon la revendication 9, dans lequel ledit composé est le 10-(3-sulfopropyl)-N-(3-sulfopropyl)-N-tosyl-9-acridinium carboxamide.

12. Composé chimiluminescent selon la revendication 1, dans lequel $R'-X^2$ est représenté par la formule :



et dans lequel $R-X^3$ est représenté par la formule :



13. Composé chimiluminescent selon la revendication 1, dans lequel ledit composé est choisi parmi les 10-méthyl-N-phényl-N-tosyl-9-acridinium carboxamide trifluorométhanesulfonate, 10-méthyl-N-phényl-N-(p-bromobenzènesulfonyl)-9-acridinium carboxamide trifluorométhanesulfonate, 10-méthyl-N-phényl-N-(p-nitrobenzènesulfonyl)-9-acridinium carboxamide trifluorométhanesulfonate, 10-méthyl-N-phényl-N-(o-nitrobenzènesulfonyl)-9-acridinium carboxamide trifluorométhanesulfonate ou 10-méthyl-N-phényl-N-trifluorométhanesulfonyl-9-acridinium carboxamide trifluorométhanesulfonate.

14. Composé chimiluminescent selon la revendication 1, dans lequel ledit composé est le 10-méthyl-N-isopropyl-N-tosyl-9-acridinium carboxamide trifluorométhanesulfonate, 10-méthyl-N-isopropyl-N-(p-bromobenzènesulfonyl)-9-acridinium carboxamide trifluorométhanesulfonate, 10-méthyl-N-isopropyl-N-(o-nitrobenzènesulfonyl)-9-acridinium carboxamide trifluorométhanesulfonate ou 10-méthyl-N-isopropyl-N-trifluorométhanesulfonyl-9-acridinium carboxamide trifluorométhanesulfonate.

15. Composé chimiluminescent selon la revendication 1, dans lequel ledit composé est le 10-méthyl-N-butyl-N-(2,4,6-triméthylbenzènesulfonyl)-9-acridinium carboxamide trifluorométhanesulfonate, 10-méthyl-N-butyl-N-(2,4,6-tri-isopropyl-benzènesulfonyl)-9-acridinium carboxamide trifluorométhanesulfonate, 10-méthyl-N-butyl-N-tosyl-9-acridinium-carboxamide trifluorométhanesulfonate, 10-méthyl-N-butyl-N-(p-bromobenzènesulfonyl)-9-acridinium carboxamide trifluorométhanesulfonate, 10-méthyl-N-butyl-N-(o-nitrophénylsulfonyl)-9-acridinium carboxamide trifluorométhanesulfonate, 10-méthyl-N-butyl-N-(p-nitrobenzènesulfonyl)-9-acridinium carboxamide trifluorométhanesulfonate, 10-méthyl-N-butyl-N-(2,4-dinitrobenzènesulfonyl)-9-acridinium carboxamide trifluorométhanesulfonate ou 10-méthyl-N-allyl-N-toxyl-9-acridinium carboxamide trifluorométhanesulfonate.

16. Composé chimiluminescent selon la revendication 1, dans lequel ledit composé est le 6-[N-toxyl-N-(2-carboxyéthyl]-phénanthridinecarboxamide, ester méthylique, 5-méthyl-6-[N-toxyl-N-(2-carboxyéthyl]-phénanthridiniumcarboxamide, ester méthylique ou 5-méthyl-6-[N-toxyl-N-(2-carboxyéthyl)]-phénanthridiniumcarboxamide.

17. Procédé de préparation d'un composé chimiluminescent comprenant les étapes de :
mettre en contact une amine représentée par la formule :

X^3-R-NH_2

avec un halogénure de sulfonyle représenté par la formule :

W-SO_2-R'-X^2

dans un solvant inerte en présence d'une base pour former un sulfonamide représenté par la formule :

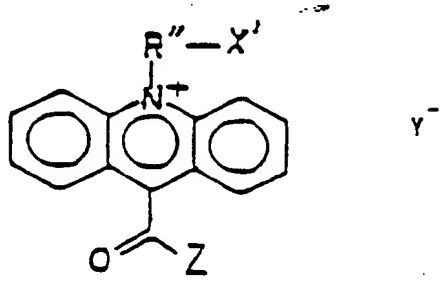
$X^3RNHSO_2R'X^2$;

et
mettre en contact le sulfonamide dans un solvant inerte en présence d'une base pour former un anion sulfonamide représenté par la formule :

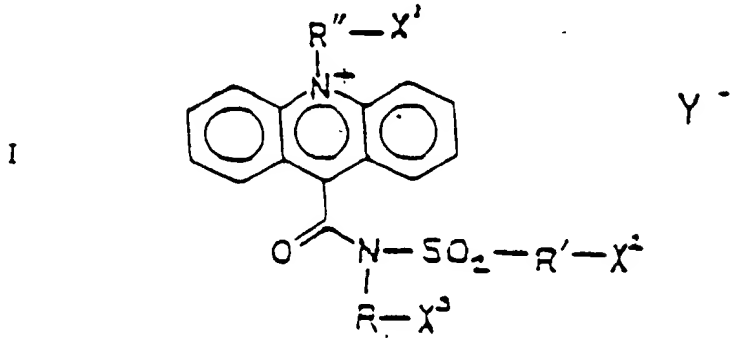
$$X^3\text{-}R\text{-}N^-\text{-}SO_2\text{-}R'\text{-}X^2 \quad M+ ;$$

et

a) acyler avec un acide 9-acridinecarboxylique activé représenté par la formule :



pour donner ledit composé chimiluminescent représenté par la formule :

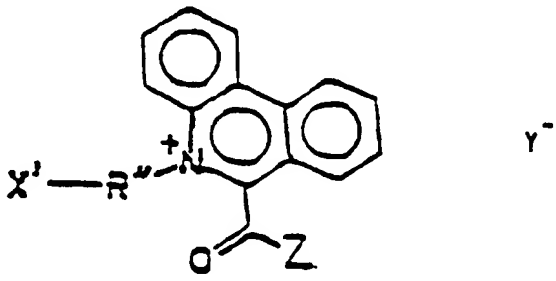


définie à la revendication 1

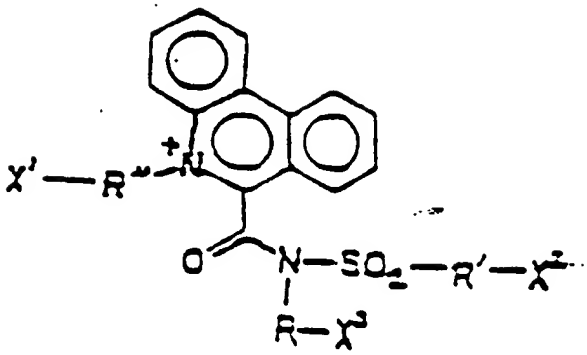
ou le 10-méthyl-N-allyl-N-p-toluènesulfonyl-9-acridinium carboxamide trifluorométhanesulfonate ;

ou

b) acyler avec un acide phénanthridine-6-carboxylique activé représenté par la formule :



pour donner I dit composé chimiluminescent représenté par la formule :

II  Y^-

définie à la revendication 1, dans laquelle W est choisi dans le groupe constitué des groupes chloro et fluoro ; et

dans laquelle M est choisi dans le groupe constitué de Li-Na et K; et

dans laquelle Z est choisi dans le groupe constitué des groupes halo, imidazolo, N-hydroxysuccinimidyle et azido.

18. Procédé de préparation d'un composé chimiluminescent comprenant les étapes de :

mettre en contact une amine représentée par la formule

X^3-R-NH_2

avec un halogénure de sulfonyle représenté par la formule :

W-SO_2-R'-X^2;

dans un solvant inerte en présence d'une base pour former un sulfonamide représenté par la formule

$X^3RNHSO_2R'X^2$;

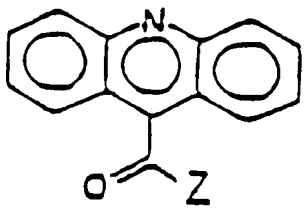
et

mettre en contact le sulfonamide dans un solvant inerte pour former un anion sulfonamide représenté par la formule :

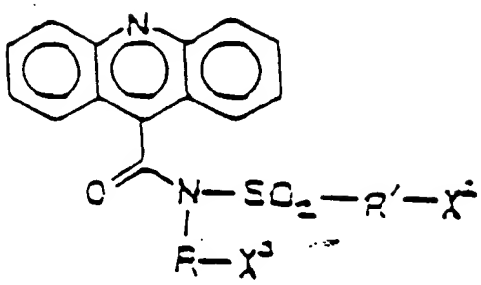
$$X^3\text{-}R\text{-}N^-\text{-}SO_2\text{-}R'\text{-}X^2 \quad M^+ ;$$

et

a) acyler avec un acide 9-acridinecarboxylique activé représenté par la formule :



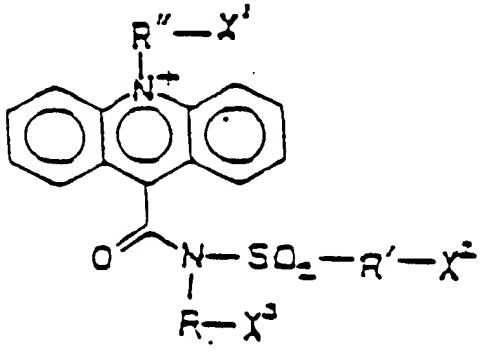
pour donner un composé représenté par la formule :



et mettre en contact ledit composé avec un agent d'alkylation de formule :

$Y\text{-}R''\text{-}X^1$

pour donner ledit composé chimiluminescent représenté par la formule :

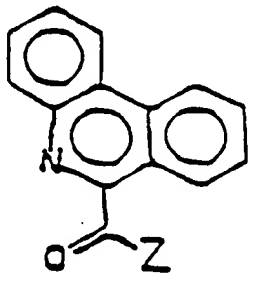
I  Y^-

définie à la revendication 1,

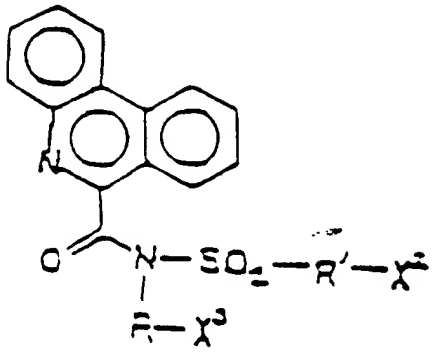
ou le 10-méthyl-N-allyl-N-p-toluènesulfonyl-9-acridinium carboxamide trifluorométhanesulfonate,

ou

b) acyler avec un acide phénanthridine-6-carboxylique activé représenté par la formule :



pour donner un composé représenté par la formule :

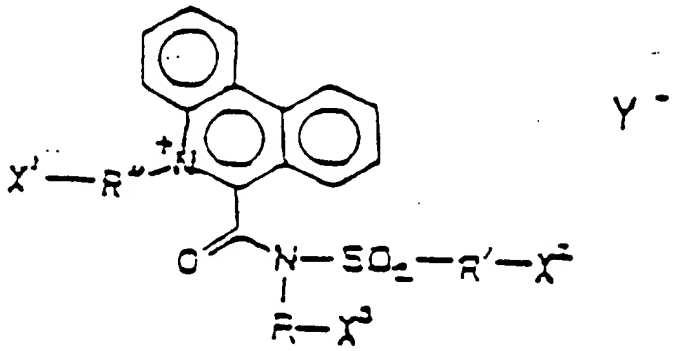


et mettre en contact ledit composé avec un agent d'alkylation de formule :

Y-R''-X^1

pour donner ledit composé chimiluminescent représenté par la formule :

II



définie à la revendication 1 ;
dans laquelle W est choisi dans le groupe constitué des groupes chloro et fluoro ; et
dans laquelle M est choisi dans le groupe constitué de Li, Na et K ; et
dans laquelle Z est choisi dans le groupe constitué des groupes halo, imidazolo, N-hydroxysuccinimidyle et azido.

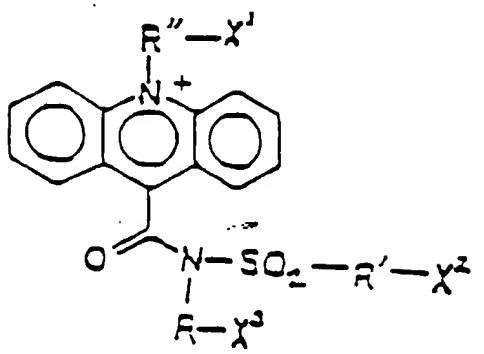
19. Procédé selon la revendication 17 ou 18, dans lequel on choisit ledit hétéroatome dans le groupe constitué de l'azote, du phosphore, du soufre et de l'oxygène.

20. Conjugué formé par un anticorps ou un antigène conjugué à un composé chimiluminescent selon la revendication 1, avec la condition supplémentaire que, dans ledit composé chimiluminescent de formule I, l'un ou l'autre des groupes X^2 et X^3 dans R'-X^2 et R-X^3 est un carboxy, carboalcoxy, carboxamido ou carboaryloxy et on choisit l'autre groupe R'-X^2 et R-X^3 parmi les hydrogène, alkyle, aryle ou benzyle ou de tels aryle ou benzyle sont substitués par un alcoxy, aryloxy, amino ou hydroxy, alors X^1 et R''-X^1 sont différents de H.

21. Procédé de réalisation d'un essai immunologique chimiluminescent pour tester la présence d'un antigène ou d'un anticorps dirigé contre un antigène selon la revendication 20 qui comprend l'étape d'exposer un échantillon à un conjugué selon la revendication 20.

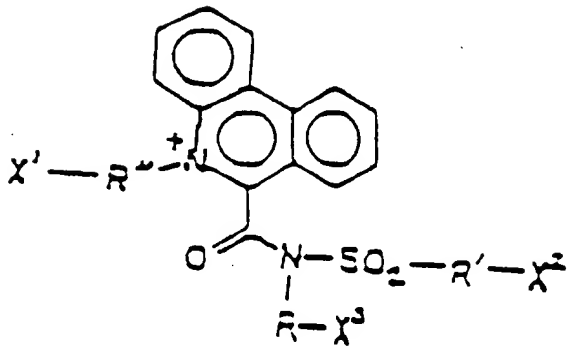
22. Conjugué formé par une sonde d'acide nucléique conjugué à un composé chimiluminescent choisi parmi les composés représentés par les formules :

I



et

II



dans lesquelles R, R' et R" peuvent comprendre indépendamment un membre choisi dans le groupe constitué des groupes alkylène, arylène, alkylène substitué et arylène substitué, de sorte que :

un ou plusieurs atomes d'hydrogène dudit membre sont remplacés par un groupe alkyle, aryle, alkyle substitué, aryle substitué, alcoxy, aryloxy, halo, amino, amino protégé, amino substitué, hydroxy, hydroxy protégé, oxo, thio, imino, mercapto ou mercapto substitué,

ou qu'un ou plusieurs atomes de carbone dudit membre sont remplacés par un hétéroatome ;

dans lesquelles X^1, X^2 et X^3 représentent indépendamment des membres du groupe constitué de l'hydrogène, des groupes carboxy, carboalcoxyle, carboxamido, carboaryloxy, cyano, carboximido, isocyanato, isothiocyanato, sulfo, halogénure de sulfonyle, halogénure de carbonyle, N-succinimidylcarboxy et N-maléimide ; ou

dans lesquelles l'un des groupes R'-X^2 ou R-X^3 peut être un nitro-benzène, pourvu que l'autre soit choisi parmi les phényle, iso-propyle, n-butyle ou benzyl-5-carboxypentyle ou dinitro-benzène, pourvu que l'autre soit choisi parmi le n-butyle et le phényle ; et

dans lesquelles Y^- est un ion approprié de signe opposé ;

à la condition que R-X^3, R'-X^2 et R"-X^1 puissent être aussi indépendamment l'hydrogène et peut être aussi le 10-méthyl-N-allyl-N-p-toluènesulfonyl-9-acridinium carboxamide trifluorométhanesulfonate.

23. Procédé de réalisation d'un essai chimiluminescent pour tester la présence d'un acide nucléique selon la revendication 22 qui comprend l'étape d'exposer un échantillon à un conjugué selon la revendication 22.

Revendications pour les Etats contractants suivants : AT, ES

1. Procédé de préparation d'un composé chimiluminescent comprenant les étapes de :

mettre en contact un amine représentée par la formule :

X^3-R-NH_2

avec un halogénure de sulfonyle représenté par la formule :

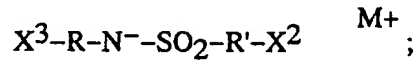
$W\text{-}SO_2\text{-}R'\text{-}X^2$

dans un solvant in rte en présence d'une base pour former un sulfonamide représenté par la formule :

$X^3RNHSO_2R'X^2$;

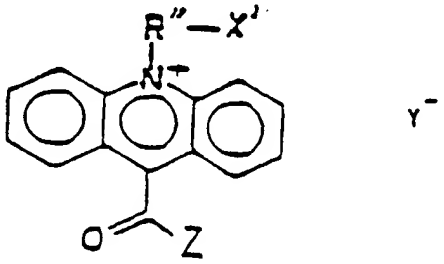
et

mettre en contact le sulfonamide dans un solvant inerte en présence d'une base pour former un anion sulfonamide représenté par la formule :



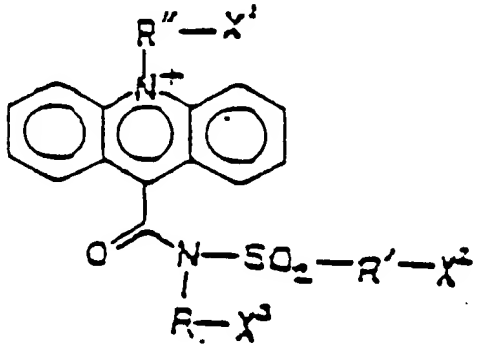
et

a) acyler avec un acide 9-acridinecarboxylique activé représenté par la formule :



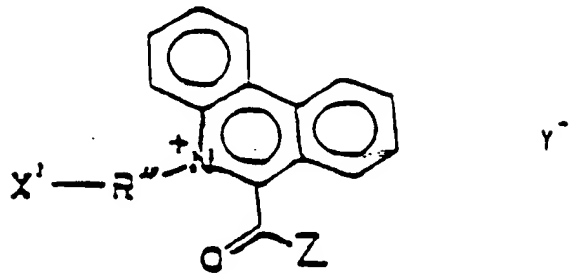
pour donner ledit composé chimiluminescent représenté par la formule :

I



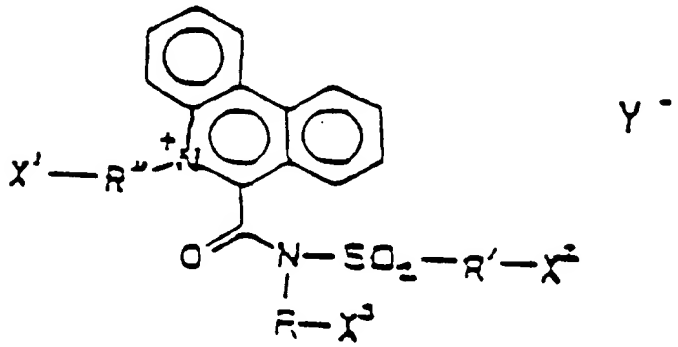
;

b) acyler avec un acide phénanthridine-6-carboxylique activé représenté par la formule :



pour donner ledit composé chimiluminescent représenté par la formule :

II



dans lesquelles R, R' et R'' représentent indépendamment un membre choisi dans le groupe constitué des groupes alkylène, arylène, alkylène substituté et arylène substitué, de sorte que :

un ou plusieurs atomes d'hydrogène dudit membre sont remplacés par un groupe alkyle, aryle, alkyle substitué, aryle substitué, alcoxy, aryloxy, halo, amino, amino protégé, amino substitué, hydroxy, hydroxy protégé, oxo, thio, imino, mercapto ou mercapto substitué,

ou qu'un ou plusieurs atomes de carbone dudit membre sont remplacés par un hétéroatome ;

dans lesquelles X^1, X^2 et X^3 représentent indépendamment des membres du groupe constitué de l'hydrogène, des groupes carboxy, carboalcoxyle, carboxamido, carboaryloxy, cyano, carboximido, isocyanato, isothiocyanato, sulfo, halogénure de sulfonyle, halogénure de carbonyle, N-succinimidylcarboxy et N-maléimide ; ou

dans lesquelles l'un des groupes R'-X^2 ou R-X^3 peut être un nitro-benzène, pourvu que l'autre soit choisi parmi les phényle, iso-propyle, n-butyle ou benzyl-5-carboxypentyle ou dinitro-benzène, pourvu que l'autre soit choisi parmi le n-butyle et phényle ; et

dans lesquelles Y^- est un ion approprié de signe opposé ;

dans lesquelles W est choisi dans le groupe constitué des groupes chlor et fluoro ; et

dans lesquelles M est choisi dans le groupe constitué de Li, Na et K ; et

dans lesquelles Z est choisi dans le groupe cosntitué des groupes halo, imidazolo, N-hydroxysuccinimidyle et azido ;

à la condition que R-X^3, R'-X^2 et R''-X^1 puissent être aussi indépendamment l'hydrogène, et

à la condition supplémentaire que, lorsque dans les composés de formule I dans l'un ou l'autre des groupes R'-X^2 et R-X^3, on choisit X^2 ou X^3 parmi les carbopentachlorophénoxy, carbo-p-nitrophénoxy, carboximido, isothiocyanate, N-maléimide et N-succinimidylcarboxy, et on choisit l'autre groupe R'-X^2 et R-X^3 parmi les hydrogène, alkyle, aryle ou benzyle, ou de tels aryl ou benzyle sont substitués par un alcoxy, aryloxy, amino ou hydroxy,

alors X^1 est différ nt de H et R''-X^1 est différent de H ;

et dans lesquelles ledit composé chimiluminescent peut être aussi le 10-méthyl-N-allyl-N-p-toluènesulfonyl-9-acridinium-carboxamide trifluorométhanesulfonate.

2. Procédé de préparation d'un composé chimilumin scent comprenant les étapes de :
mettre en contact une amine représentée par la formule :

X^3-R-NH_2

avec un halogénure de sulfonyle représenté par la formule :

W-SO_2-R'-X^2

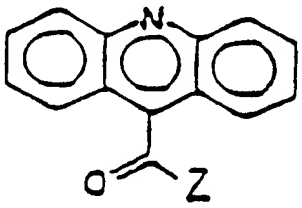
dans un solvant inerte en présence d'une base pour former un sulfonamide représenté par la formule :

$X^3RNHSO_2R'X^2$;

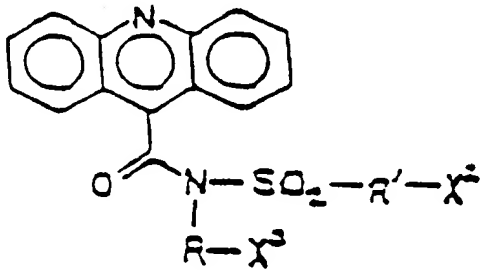
et
mettre en contact le sulfonamide dans un solvant inerte en présence d'une base pour former un anion sulfonamide représenté par la formule :

$$X^3\text{-R-}N^-\text{-}SO_2\text{-R'-}X^2 \quad M^+ ;$$

et
a) acyler avec un acide 9-acridinecarboxylique activé représenté par la formule :



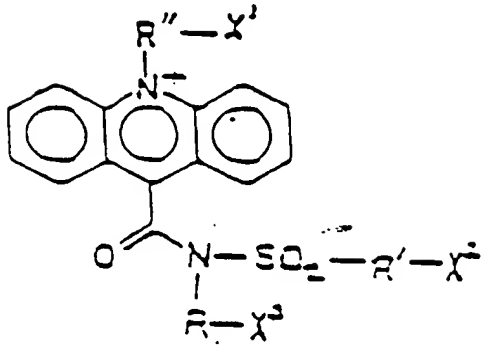
pour donner ledit composé chimiluminescent représenté par la formule :



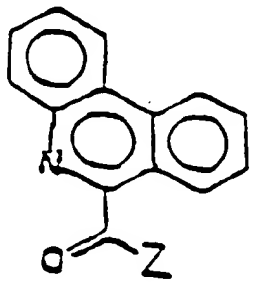
et mettre en contact ledit composé avec un agent d'alkylation de formule :

Y-R''-X^1

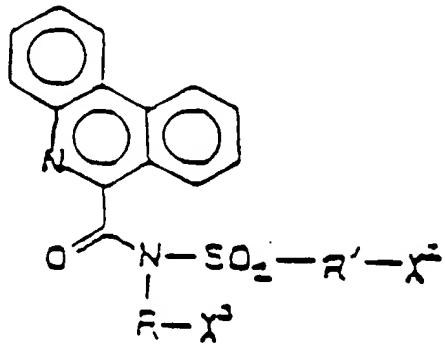
pour donner ledit composé chimiluminescent représenté par la formule :

I  ;

b) acyler avec un acide phénanthridine-6-carboxylique activé représenté par la formule :



pour donner un composé représenté par la formule :

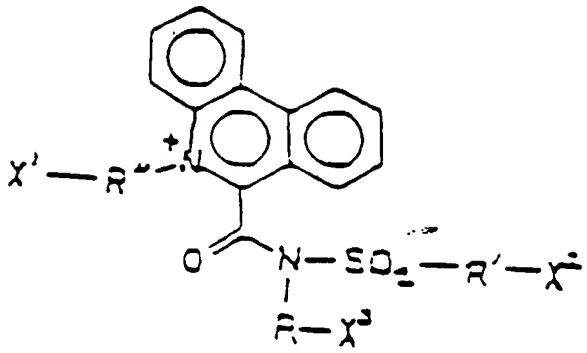


et mettre en contact ledit composé avec un agent d'alkylation de formule :

$Y\text{-}R''\text{-}X^1$

pour donner ledit composé chimiluminescent représenté par la formule :

II



dans lesquelles R, R' et R'' représentent indépendamment un nombre choisi dans le groupe constitué des groupes alkylène, arylène, alkylène substitué et arylène substitué, de sorte que : un ou plusieurs atomes d'hydrogène dudit membre sont remplacés par un groupe alkyle, aryle, alkyle substitué, aryle substitué, alcoxy, aryloxy, halo, amino, amino protégé, amino substitué, hydroxy, hyroxy protégé, oxo, thio, imino, mercapto ou mercapto substitué,

ou qu'un ou plusieurs atomes de carbone dudit membre sont remplacés par un hétéroatome ;

dans lesquelles X^1, X^2 et X^3 représentent indépendamment des membres du groupe constitué de l'hydrogène, des groupes carboxy, carboalcoxyle, carboxamido, carboaryloxy, cyano, carboximido, isocyanato, isothiocyanato, sulfo, halogénure de sulfonyle, halogénure de carbonyle, N-succinimidylcarboxy et N-maléimide ; ou

dans lesquelles l'un des groupes $R'-X^2$ ou $R-X^3$ peut être un nitro-benzène, pourvu que l'autre soit choisi parmi les phényle, iso-propyle, n-butyle ou benzyl-5-carboxypentyle ou dinitro-benzène, pourvu que l'autre soit choisi parmi le n-butyle et le phényle ; et

dans lesquelles Y^- est un ion approprié de signe opposé ;

dans lesquelles W est choisi dans le groupe constitué des groupes chloro et fluoro ; et

dans lesquelles M est choisi dans le groupe constitué de Li, Na et K ; et

dans lesquelles Z est choisi dans le groupe cosntitué des groupes halo, imidazolo, N-hydroxysuccinimidyle et azido ;

à la condition que $R-X^3$, $R'-X^2$ et $R''-X^1$ puissent être aussi indépendamment l'hydrogène, et

à la condition supplémentaire que, lorsque dans les composés de formule I dans l'un ou l'autre des groupes $R'-X^2$ et $R-X^3$, on choisit X^2 ou X^3 parmi les carbopentachlorophénoxy, carbo-p-nitrophénoxy, carboximido, isothiocyanate, N-maléimide et N-succinimidylcarboxy, et on choisit l'autre groupe $R'-X^2$ et $R-X^3$ parmi les hydrogène, alkyle, aryle ou benzyle, ou de tels aryle ou benzyle sont substitués par un alcoxy, aryloxy, amino ou hydroxy,

alors X^1 est différent de H et $R''-X^1$ est différent de H ;

et dans lesquelles ledit composé chimiluminescent peut être aussi le 10-méthyl-N-allyl-N-p-toluènesulfonyl-9-acridinium-carboxamide trifluorométhanesulfonate.

3. Procédé selon la revendication 1 ou 2, dans lequel on choisit ledit hétéroatome dans le groupe constitué de l'azote, du phosphore, du soufre et de l'oxygène.

4. Procédé selon la revendication 1 ou 2, dans lequel ledit composé est le 10-méthyl-N-[2-carboxyéthyl]-N-tosyl-9-acridinium carboxamide.

5. Procédé selon la revendication 1 ou 2, dans lequel ledit composé est le 10-méthyl-N-(4-carboxybutyl)-N-tosyl-9-acridinium carboxamide.

6. Procédé selon la revendication 1 ou 2, dans lequel ledit composé est le 10-méthyl-N-(5-carboxypentyl)-N-tosyl-9-acridinium carboxamide.

7. Procédé selon la r vendication 1 ou 2, dans lequel ledit composé est le 10-(3-sulfopropyl)-N-(2-carboxyéthyl)-N-tosyl-9-acridinium carboxamide.

8. Procédé selon la revendication 1 ou 2, dans lequel ledit composé est le 10-(3-sulfopropyl)-N-(3-sulfopropyl)-N-tosyl-9-acridinium carboxamide.

9. Procédé selon la revendication 1 ou 2, dans lequel ledit composé est choisi parmi les 10-méthyl-N-phényl-N-tosyl-9-acridinium carboxamide trifluorométhanesulfonate, 10-méthyl-N-phényl-N-(p-bromobenzènesulfonyl)-9-acridinium carboxamide trifluorométhanesulfonate, 10-méthyl-N-phényl-N-(p-nitrobenzènesulfonyl)-9-acridinium carboxamide trifluorométhanesulfonate, 10-méthyl-N-phényl-N-(o-nitrobenzènesulfonyl)-9-acridinium carboxamide trifluorométhanesulfonate ou 10-méthyl-N-phényl-N-trifluorométhanesulfonyl-9-acridinium carboxamide trifluorométhanesulfonate.

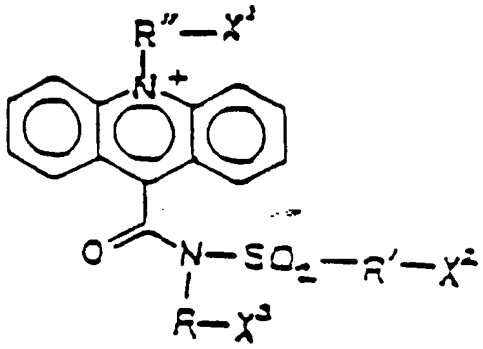
10. Procédé selon la revendication 1 ou 2, dans lequel ledit composé est le 10-méthyl-N-isopropyl-N-tosyl-9-acridinium carboxamide trifluorométhanesulfonate, 10-méthyl-N-isopropyl-N-(p-bromobenzènesulfonyl)-9-acridinium carboxamide trifluorométhanesulfonate, 10-méthyl-N-isopropyl-N-(o-nitrobenzènesulfonyl)-9-acridinium carboxamide trifluorométhanesulfonate ou 10-méthyl-N-isopropyl-N-trifluorométhanesulfonyl-9-acridinium carboxamide trifluorométhanesulfonate.

11. Procédé selon la revendication 1 ou 2, dans lequel ledit composé est le 10-méthyl-N-butyl-N-(2,4,6-triméthylbenzènesulfonyl)-9-acridinium carboxamide trifluorométhanesulfonate, 10-méthyl-N-butyl-N-(2,4,6-triisopropyl-benzènesulfonyl)-9-acridinium carboxamide trifluorométhanesulfonate, 10-méthyl-N-butyl-N-toxyl-9-acridinium carboxamide trifluorométhanesulfonate, 10-méthyl-N-butyl-N-(p-bromobenzènesulfonyl)-9-acridinium carboxamide trifluorométhanesulfonate, 10-méthyl-N-butyl-N-(o-nitrophénylsulfonyl)-9-acridinium carboxamide trifluorométhanesulfonate, 10-méthyl-N-butyl-N-(p-nitrobenzènesulfonyl)-9-acridinium carboxamide trifluorométhanesulfonate, 10-méthyl-N-butyl-N-(2,4-dinitrobenzènesulfonyl)-9-acridinium carboxamide trifluorométhanesulfonate ou 10-méthyl-N-allyl-N-toxyl-9-acridinium carboxamide trifluorométhanesulfonate.

12. Procédé selon la revendication 1 ou 2, dans lequel ledit composé est le 6-[N-toxyl-N-(2-carboxyéthyl]-phénanthridinecarboxamide, ester méthylique, 5-méthyl-6-[N-tosyl-N-(2-carboxyéthyl]-phénanthridiniumcarboxamide, ester méthylique, ou 5-méthyl-6-[N-toxyl-N-(2-carboxyéthyl)]-phénanthridiniumcarboxamide.

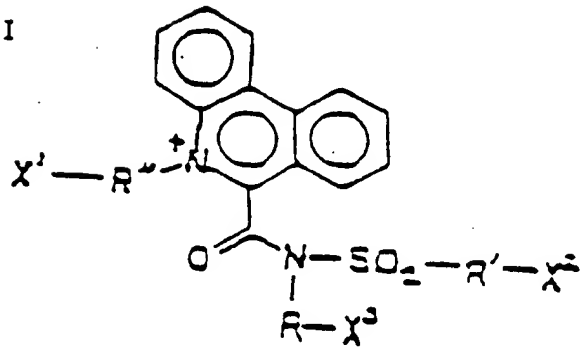
13. Procédé de réalisation d'un essai immunologique pour tester la présence d'un antigène ou d'un anticorps dirigé contre un antigène qui comprend l'étape d'exposer un échantillon à un conjugué formé d'un anticorps d'un antigène conjugué à un composé chimiluminescent choisi parmi les composés représentés par les formules :

I



et

II



dans lesquelles R, R' et R'' représentent indépendamment un nombre choisi dans le groupe constitué des groupes alkylène, arylène, alkylène substituté et arylène substitué, de sorte que :

un ou plusieurs atomes d'hydrogène dudit membre sont remplacés par un groupe alkyle, aryle, alkyle substitué, aryle substitué, alcoxy, aryloxy, halo, amino, amino protégé, amino substitué, hydroxy, hydroxy protégé, oxo, thio, imino, mercapto ou mercapto substitué,

ou qu'un ou plusieurs atomes de carbone dudit membre sont remplacés par un hétéroatome ;

dans lesquelles X^1, X^2 et X^3 représentent indépendamment des membres du groupe constitué de l'hydrogène, des groupes carboxy, carboalcoxyle, carboxamido, carboaryloxy, cyano, carboximido, isocyanato, isothiocyanato, sulfo, halogénure de sulfonyle, halogénure de carbonyle, N-succinimidylcarboxy et N-maléimide ; ou

dans lesquelles l'un des groupes $R'-X^2$ ou $R-X^3$ peut être un nitro-benzène, pourvu que l'autre soit choisi parmi les phényle, iso-propyle, n-butyle ou benzyl-5-carboxypentyle ou dinitro-benzène, pourvu que l'autre soit choisi parmi le n-butyle et le phényle ; et

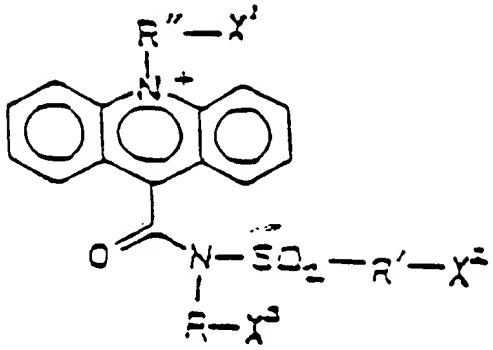
dans lesquelles Y^- est un ion approprié de signe opposé ;

à la condition que $R-X^3$, $R'-X^2$ et $R''-X^1$ puissent être aussi indépendamment l'hydrogène, et à la condition supplémentaire que, lorsque dans les composés de formule I dans l'un ou l'autre des groupes $R'-X^2$ et $R-X^3$, on choisit X^2 ou X^3 parmi les carbopentachlorophénoxy, carbo-p-nitrophénoxy, carboximido, isothiocyanate, N-maléimide et N-succinimidylcarboxy, et on choisit l'autre groupe $R'-X^2$ et $R-X^3$ parmi les hydrogène, alkyle, aryle ou benzyle, ou de tels aryle ou benzyle sont substitués par un alcoxy, aryloxy, amino ou hydroxy,

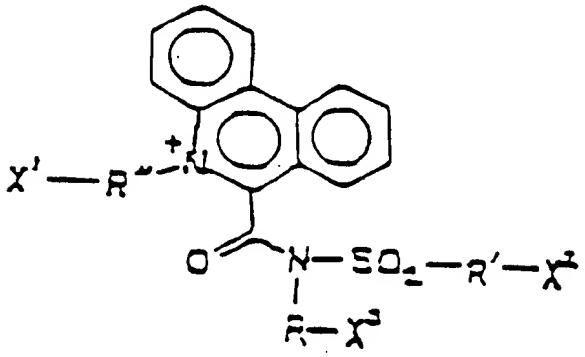
alors X^1 est différent de H et $R''-X^1$ est différent de H ;

et peut être aussi le 10-méthyl-N-allyl-N-p-toluènesulfonyl-9-acridinium-carboxamide trifluorométhanesulfonate.

14. Procédé de réalisation d'un essai immunologique pour tester la présence d'un acide nucléique qui comprend l'étape d'exposer un échantillon à un conjugué par une sonde d'acide nucléique conjuguée à un composé chimiluminescent choisi parmi les composés représentés par les formules :

I  Y^-

et

II  Y^-

dans lesquelles R, R' et R'' représentent indépendamment un membre choisi dans le groupe constitué des groupes alkylène, arylène, alkylène substituté et arylène substitué, de sorte que :

un ou plusieurs atomes d'hydrogène dudit membre sont remplacés par un groupe alkyle, aryle, alkyle substitué, aryle substitué, alcoxy, aryloxy, halo, amino, amino protégé, amino substitué, hydroxy, hydroxy protégé, oxo, thio, imino, mercapto ou mercapto substitué,

ou qu'un ou plusieurs atomes de carbone dudit membre sont remplacés par un hétéroatome ;

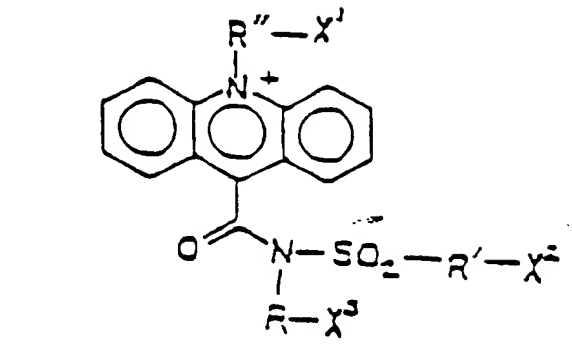
dans lesquelles X^1, X^2 et X^3 représentent indépendamment des membres du groupe constitué de l'hydrogène, des groupes carboxy, carboalcoxyle, carboxamido, carboaryloxy, cyano, carboximido, isocyanato, isothiocyanato, sulfo, halogénure de sulfonyle, halogénure de carbonyle, N-succinimidylcarboxy et N-maléimide ; ou

dans lesquelles l'un des groupes $R'-X^2$ ou $R-X^3$ peut être un nitro-benzène, pourvu que l'autre soit choisi parmi les phényle, iso-propyle, n-butyle ou benzyl-5-carboxypentyle ou dinitro-benzène, pourvu que l'autre soit choisi parmi le n-butyle et la phényle ; et

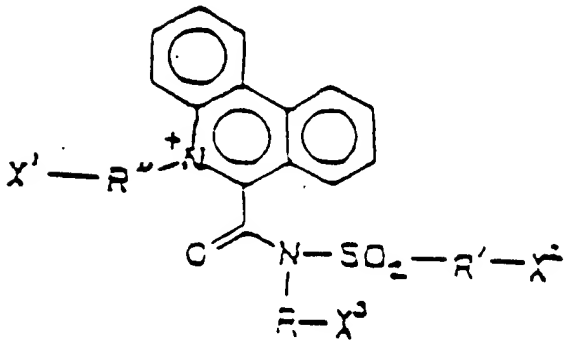
dans lesquelles Y^- est un ion approprié de signe opposé ;

à la condition que $R-X^3$, $R'-X^2$ et $R''-X^1$ puissent être aussi indépendamment l'hydrogène et peut être aussi le 10-méthyl-N-allyl-N-p-toluènesulfonyl-9-acridinium carboxamide trifluorométhanesulfonate.

15. Procédé de préparation d'un conjugué d'anticorps ou d'antigène d'un composé chimiluminescent qui comprend les étapes de coupler de manière covalente un anticorps ou un antigène à un composé chimiluminescent choisi parmi les composés représentés par les formules :

I  Y^-

et

II  Y^-

dans lesquelles R, R' et R'' représentent indépendamment un nombre choisi dans le groupe constitué des groupes alkylène, arylène, alkylène substituté et arylène substitué, de sorte que :

un ou plusieurs atomes d'hydrogène dudit membre sont remplacés par un groupe alkyle, aryle, alkyle substitué, aryle substitué, alcoxy, aryloxy, halo, amino, amino protégé, amino substitué, hydroxy, hydroxy protégé, oxo, thio, imino, mercapto ou mercapto substitué,

ou qu'un ou plusieurs atomes de carbone dudit membre sont remplacés par un hétéroatome ;

dans lesquelles X^1, X^2 et X^3 représentent indépendamment des membres du groupe constitué de l'hydrogène, des groupes carboxy, carboalcoxyle, carboxamido, carboaryloxy, cyano, carboximido, isocyanato, isothiocyanato, sulfo, halogénure de sulfonyle, halogénure de carbonyle, N-succinimidylcarboxy et N-maléimide ; ou

dans lesquelles l'un des groupes $R'-X^2$ ou $R-X^3$ peut être un nitro-benzène, pourvu que l'autre soit choisi parmi les phényle, iso-propyle, n-butyle ou benzyl-5-carboxypentyle ou dinitro-benzène, pourvu que l'autre soit choisi parmi le n-butyle et la phényle ; et

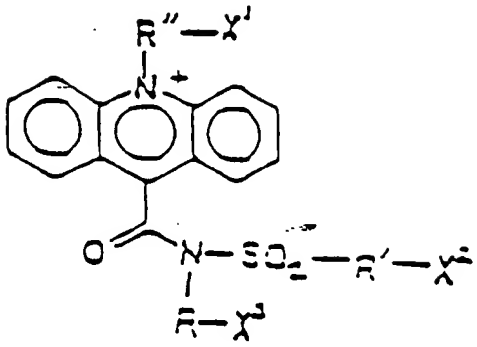
dans lesquelles Y^- est un ion approprié de signe opposé ;

à la condition que $R-X^3$, $R'-X^2$ et $R''-X^1$ puissent être aussi indépendamment l'hydrogène, et à la condition supplémentaire que, lorsque dans les composés de formule I dans l'un ou l'autre des groupes $R'-X^2$ et $R-X^3$, on choisit X^2 ou X^3 parmi les carbopentachlorophénoxy, carbo-p-nitrophénoxy, carboximido, isothiocyanate, N-maléimide et N-succinimidylcarboxy, et on choisit l'autre groupe $R'-X^2$ et $R-X^3$ parmi les hydrogène, alkyle, aryle ou benzyle, ou de tels aryle ou benzyle sont substitués par un alcoxy, aryloxy, amino ou hydroxy,

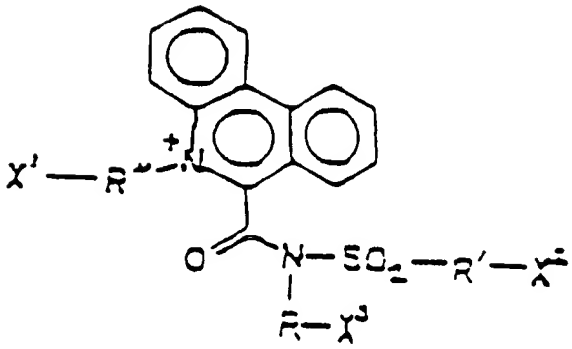
alors X^1 est différent de H et $R''-X^1$ est différent de H ;

et peut être aussi le 10-méthyl-N-allyl-N-p-toluènesulfonyl-9-acridinium-carboxamide trifluorométhanesulfonate.

16. Procédé de préparation d'un conjugué d'une sonde d'acide nucléique et d'un composé chimiluminescent qui comprend les étapes de coupler de manière covalente un composé chimiluminescent choisi parmi les composés représentés par les formules :

I  Y^-

et

II  Y^-

dans lesquelles R, R' et R'' représentent indépendamment un membre choisi dans le groupe constitué des groupes alkylène, arylène, alkylène substituté et arylène substitué, de sorte que :

un ou plusieurs atomes d'hydrogène dudit membre sont remplacés par un groupe alkyle, aryle, alkyle substitué, aryle substitué, alcoxy, aryloxy, halo, amino, amino protégé, amino substitué, hydroxy, hydroxy protégé, oxo, thio, imino, mercapto ou mercapto substitué,

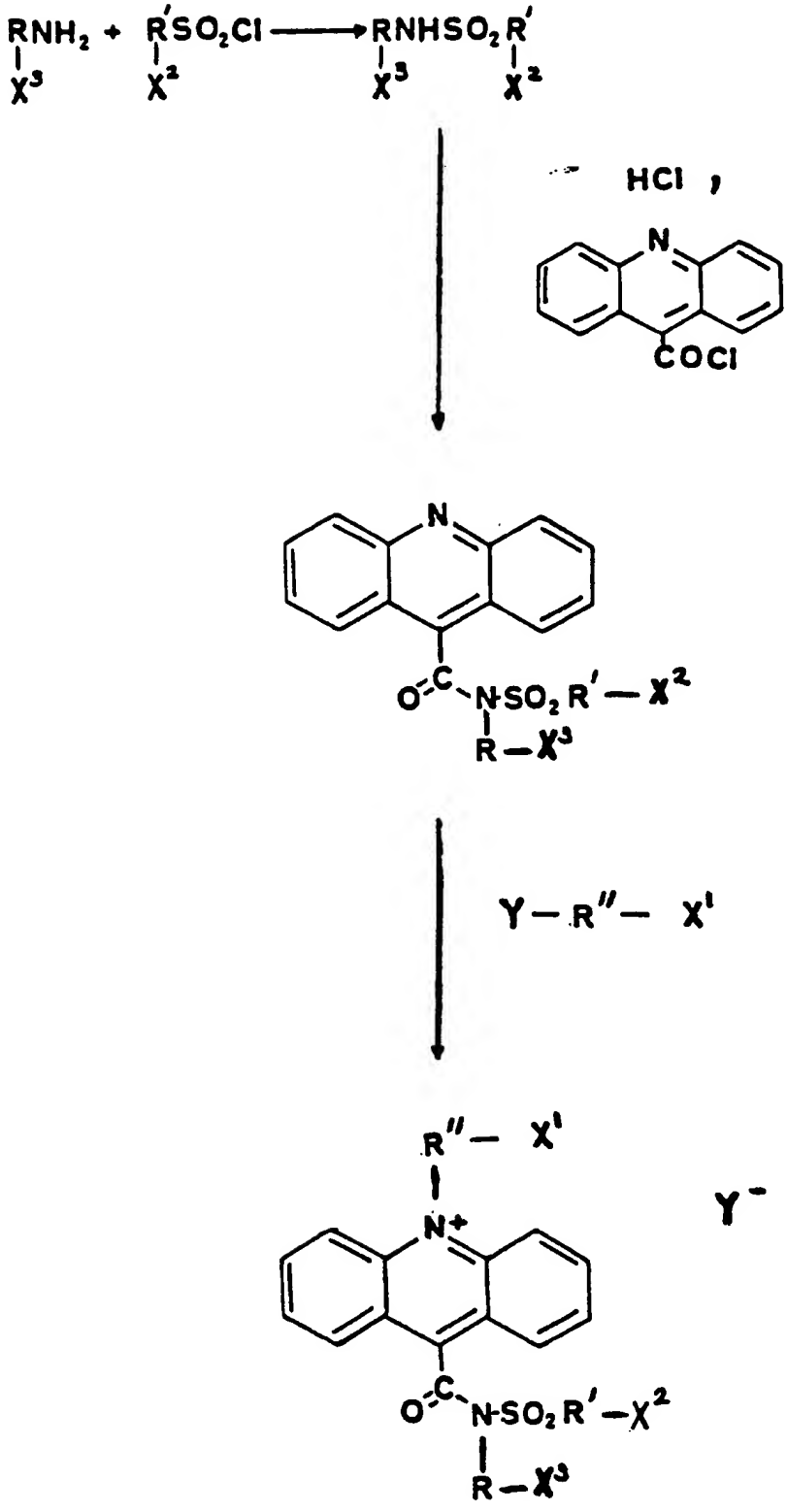
ou qu'un ou plusieurs atomes de carbone dudit membre sont remplacés par un hétéroatome ;

dans lesquelles X^1, X^2 et X^3 représentent indépendamment des membres du groupe constitué de l'hydrogène, des groupes carboxy, carboalcoxyle, carboxamido, carboaryloxy, cyano, carboximido, isocyanato, isothiocyanato, sulfo, halogénure de sulfonyle, halogénure de carbonyle, N-succinimidylcarboxy et N-maléimide ; ou

dans lesquelles l'un des groupes R'-X^2 ou R-X^3 peut être un nitro-benzène, pourvu que l'autre soit choisi parmi les phényle, iso-propyle, n-butyle ou benzyl-5-carboxypentyle ou dinitro-benzène, pourvu que l'autre soit choisi parmi le n-butyle et la phényle ; et

dans lesquelles Y^- est un ion approprié de signe opposé ;

à la condition que R-X^3, R'-X^2 et R''-X^1 puissent être aussi indépendamment l'hydrogène et peut être aussi le 10-méthyl-N-allyl-N-p-toluènesulfonyl-9-acridinium carboxamide trifluorométhanesulfonate.


RNH2 + R'SO2Cl → RNHSO2R'
X3 X2 X3 X2
HCl ,
N
COCl
N
O=C
N-SO2R'—X2
R—X3
Y—R''— X1
R''— X1
N+
Y-
O=C
N-SO2R'—X2
R—X3